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CORRIGENDA

No. CLXXVII, January 1931.

P. 28. *In the last line for other type read upper.*

204. *The last three lines should read: This difference in the obliquity of the starch cells occurs specifically where it might be expected on general grounds of affinity, since there is little doubt but that . . .*

ADDENDUM

No. CLXXVIII, April 1931.

P. 211. *Add following footnote.* Studies in the Cytology of the Hibiscus III is a continuation of the thesis approved for the Degree of Doctor of Science in the University of London, the first part of which appeared as Study II in No. CLXXVII (January 1931).

The Sexuality of the Normal, Giant, and Dwarf Spores of *Pleurage anserina* (Ces.), Kuntze.

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With Plate I and ten Figures in the Text.

I. INTRODUCTION.

PLEURAGE is a genus of the Pyrenomycetes included in the coprophilous order Fimetariales. The ascospores bear gelatinous hyaline appendages (Text-figs. 2 and 3), a character which distinguishes *Pleurage* from related genera. The asci of the majority of the species contain eight spores, but there are several species in which the asci contain only four spores. In North America there are five four-spored species (7), among which is *P. anserina* (Pl. I, Fig. 1).

Shear and Dodge in 1927 (8), B. O. Dodge, 1927-30 (2, 3, 4), and Margaret Wilcox in 1929 (9) have investigated the sexual phenomena in species of *Neurospora* from the experimental point of view, and Dodge has succeeded in obtaining fertile hybrids from crosses between *N. sitophila* and *N. tetrasperma*.

N. sitophila has eight spores in each ascus and *N. tetrasperma* usually four spores in each ascus. Dodge and his co-workers have come to the following conclusions in respect to sex in these two species:

(1) In *N. sitophila* the eight spores in each ascus are uninucleate and unisexual. When grown separately, a mycelium of monosporous origin does not fruit. Fruit-bodies are produced only when monosporous mycelia are suitably mated. In each ascus four spores are of one sex and four of the opposite sex.

(2) In *N. tetrasperma* the four spores normally present in each ascus are binucleate and bisexual. When grown separately, a mycelium of monosporous origin fruits readily. The spores, from the sexual point of view, are all alike, in that they are bisexual.

(3) In *N. tetrasperma*, occasionally, an ascus contains one or more spores of *giant* or *dwarf* size. A single giant spore replaces two or even four normal spores and probably contains more than two nuclei. Cultural

experiments indicate that giant spores are bisexual. Two dwarf spores replace a single normal spore, and each dwarf spore contains only one nucleus. Cultural experiments indicate that dwarf spores are all unisexual.

(4) Sexuality of the normal spores of *N. tetrasperma* is due to the presence of two nuclei of opposite sex in each spore. When two dwarf spores replace a normal spore these two nuclei are separated, and presumably the two spores in each dwarf pair are of opposite sex.

Shear and Dodge (8), when making their investigations on *N. tetrasperma*, did not extract a pair of dwarf spores from any ascus containing them, and therefore were unable to determine: (1) whether or not the two members of each pair of dwarf spores are of opposite sex; and (2) whether or not, if they are of opposite sex, the mycelia to which they give rise are compatible with one another in that they can mate and produce fruit-bodies.

With a view to throwing further light on sexual phenomena concerned with normal, giant, and dwarf spores in Pyrenomycetes with four-spored asci the writer undertook to investigate *P. anserina*.

II. MATERIAL AND METHODS.

P. anserina has black pyriform perithecia with a papilliform or slightly cylindric beak which is usually curved. At Winnipeg the fungus commonly appears on horse-dung cultures in the laboratory, and many perithecia can often be observed set close together at the surface of a single horse-dung ball (Pl. I, Fig. 1).

A spore-deposit of *P. anserina* can be readily obtained by placing a glass slide about three-quarters of an inch above perithecia on horse-dung. The spores are shot from the perithecia on to the under side of the slide, to which they remain attached.

It was found, by observing with the microscope, that the spore-deposits of *P. anserina* are composed almost entirely of *normal* spores of rather uniform size (average $20 \times 40 \mu$), but that here and there in a deposit there occur *giant* and *dwarf* spores (Pl. I, Fig. 4) similar to those described by Dodge for *N. tetrasperma*.

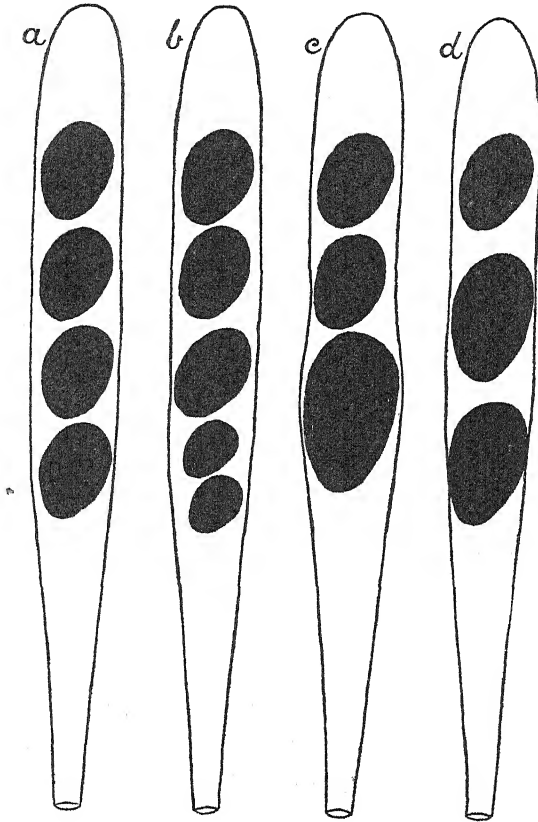
Giant spores of *P. anserina*, in volume, are one and one-half times or twice as large as normal spores (Text-fig. 5); and they are but rarely produced, for only about one spore in a thousand is a giant.

Dwarf spores of *P. anserina*, in volume, are half as large as normal spores (Text-fig. 5), and they are produced more frequently than giant spores, for about one spore in every two hundred is a dwarf.

On examining intact asci, it was found that a giant spore may replace two normal spores (cf. *a* and *c* in Text-fig. 1), or that two giant spores may replace three normal spores (cf. *a* and *d* in Text-fig. 1); and it was

also found that the dwarf spores always occur in pairs, and that each pair of dwarf spores replaces a single normal spore (cf. *a* and *b* in Text-fig. 1).

In *N. tetrasperma*, when the four spores are shot out from an ascus, they separate from one another and so become widely scattered in the



TEXT-FIG. 1. *Pleurage anserina*. Diagram of asci containing different numbers of spores : *a*, usual type of ascus with four normal spores; *b*, five-spored ascus containing three normal spores and two dwarf spores which have replaced one normal spore; *c*, three-spored ascus containing two normal spores and one giant spore which has replaced two normal spores; and *d*, three-spored ascus, containing one normal spore and two giant spores which have replaced three normal spores.

spore-deposit. In *P. anserina*, on the other hand, when four spores are shot out from an ascus, owing to their possessing mucilaginous appendages they tend to cling together at the time of, and after, their discharge, with the result that they are usually deposited together in a mass or string on any slide which receives them (Pl. I, Fig. 5, 6, and 7).

When a pair of dwarf spores is shot out from an ascus of *N. tetrasperma*, the two spores separate from one another and cannot be recognized as a pair in the spore-deposit; but, when a pair of dwarf spores is shot out

from an ascus of *P. anserina*, the two spores usually cling together so that they are found close together in the spore-deposit. Three normal spores and a pair of dwarf spores shot out from one and the same ascus are shown as they appeared in a dry spore-deposit in Text-fig. 4. The appendages were not clearly visible as the deposit had dried, and they therefore have not been represented. Appendages can be seen best when the spore-deposit is moist, but they are faintly visible in the dry spore-deposits illustrated in Pl. I, Figs. 5 and 7.

In order to obtain monosporous mycelia, spores were removed from a spore-deposit singly on the point of a dry needle (5) and transferred to a sterilized culture medium.

Using the dry-needle method of picking up spores it is possible to remove and sow in a culture medium all the four spores of an ascus (cf. Text-fig. 3) in order, beginning with the uppermost and proceeding to the lowest. The four spores of twenty normal asci were thus sown. Of ascus spore-deposits like that shown in Text-fig. 4 (three normal spores and two dwarf spores) five were sown in a similar manner; and there were also sown a number of other pairs of dwarf spores picked out of various general spore-deposits.

When spores were sown on horse-dung agar, in most trials the spores which germinated were only about 6 per cent. of the whole number, while in other trials as many as 50 per cent. germinated. This relatively low percentage of germination was unsatisfactory for the needs of the work. Dodge (1) found that heating the spores of certain Ascomycetes often effectively increases their power to germinate. The spores of *P. anserina* were therefore slowly warmed up to 25°, 30°, 35°, 40°, and 45° C. and then cooled; but the percentage of germination was not increased. Dung agar as a culture medium was then abandoned.

It was found that the spores germinate very well: (1) in a hanging drop of sterile water to which a few straws of sterile dung have been added; (2) in a hanging drop of dung-water made by stirring up horse-dung and water and then sterilizing the decanted liquid; and (3) on fresh sterilized horse-dung (Pl. I, Figs. 1, 2, and 3). When these methods were employed, the number of spores which germinated was 75–95 per cent.

There is no difficulty in obtaining perithecia on monosporous mycelia derived from normal spores. The perithecia develop within two weeks after the spores have been sown provided that the culture medium consists of fresh sterilized horse-dung and that the culture medium is kept sufficiently moist, is exposed to the light, and is maintained at room temperature (about 22° C.).

Some experiments made to determine the effect of light and of high temperature upon the fruiting of the mycelia of *P. anserina* will now be recorded.

Of four monosporous mycelia derived from normal spores and grown on horse-dung (cf. Pl. I, Fig. 1) two were set in the light and two were kept in the dark. At the end of eight days, the illuminated cultures began to develop perithecia; whereas, at the end of twenty days, the cultures kept in the dark showed no signs of fruiting. At the end of twenty days, the cultures which had been kept in the dark were exposed to daylight and, at the end of ten days after thus becoming illuminated, they began to develop perithecia. From these experiments it appears that the mycelia of *P. anserina* develop perithecia only when exposed to light.

Of four monosporous mycelia derived from normal spores and grown on horse dung (cf. Pl. I, Fig. 1) two were subjected to a high temperature, namely, 35° C., and the other two were kept at room temperature, about 22° C. At the end of seven days, the cultures kept at room temperature began to develop perithecia; whereas the two cultures subjected to a temperature of 35° C. showed no signs of fruiting. At the end of nineteen days, the cultures which had been kept at 35° C. were set on a table in a room at a temperature of about 22° C. and, at the end of nine more days, they began to develop perithecia. From these experiments we may conclude that the development of perithecia is prevented by a high temperature.

III. THE SEXUALITY OF THE NORMAL SPORES.

As a first step in an attempt to determine the sexuality of the normal spores of *P. anserina*, single spores were planted in hanging drops of dung decoction, and those that germinated were transferred to horse-dung. By this method eighteen monosporous mycelia were obtained.

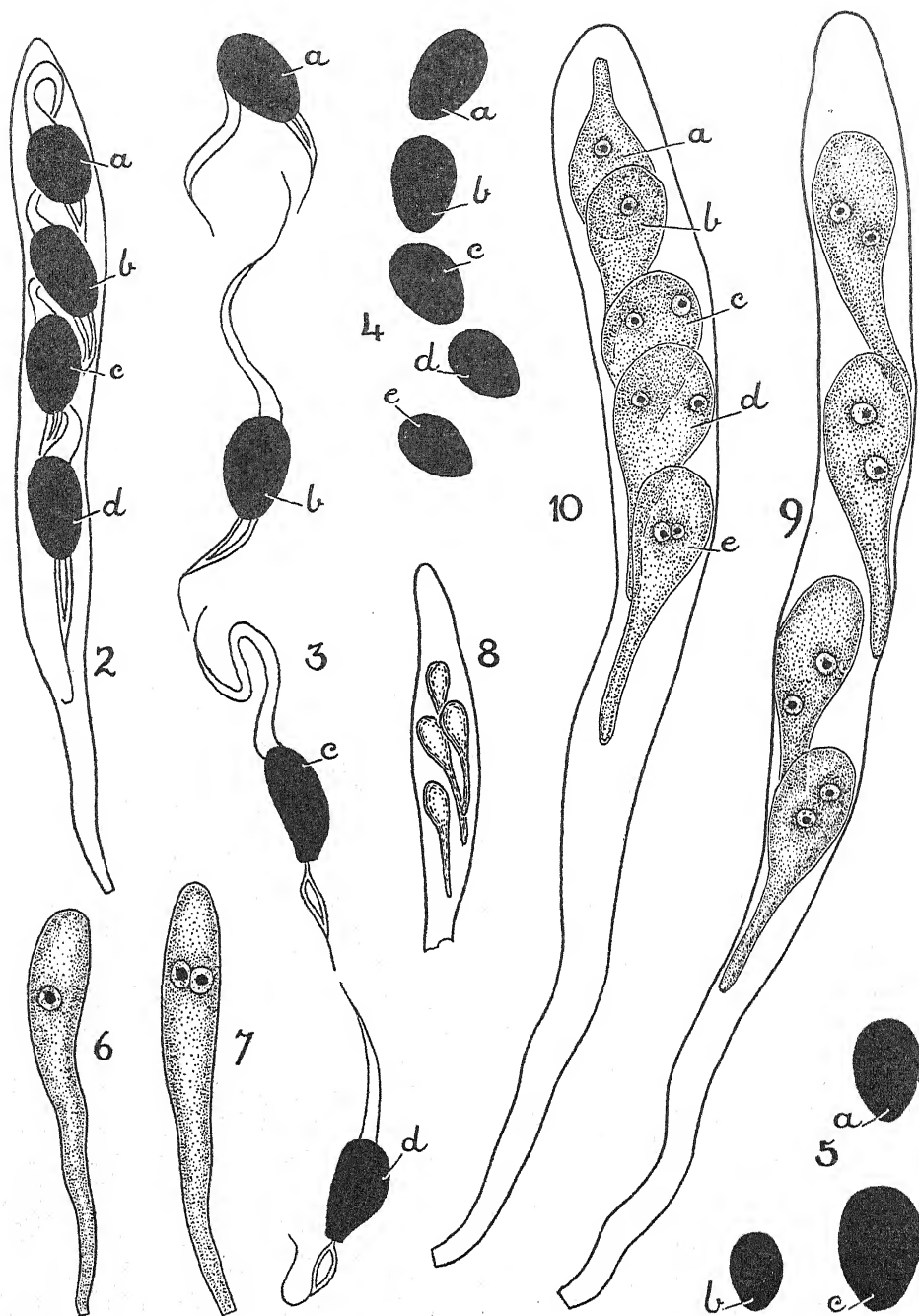
Each spore, within three days of being sown, developed a vigorous mycelium. The mycelia bore neither oidia nor any other kind of secondary spore which might be a source of contamination of the cultures. Special care is necessary in culturing fungi with secondary spores. Thus Dodge, when transferring the spores of *N. tetrasperma*, was obliged to take special precautions against including any conidia with them.

At the end of a week, the eighteen monosporous cultures began to fruit, and by the end of two weeks all of them had fruited (Pl. I, Fig. 1). Subsequently, nearly a hundred other normal spores were separately germinated, and the monosporous mycelia which resulted all fruited when they were kept under suitable conditions.

The observations just recorded indicate that the normal spores of *P. anserina* are bisexual.

IV. THE SEXUALITY OF THE GIANT SPORES.

Monosporous cultures were made of six giant spores, each twice the size of a normal spore. Three of these spores germinated and, after two



TEXT-FIGS. 2-10. *Pleurage anserina*. Camera-lucida drawings of asci and ascospores. Fig. 2. Four spores, *a*, *b*, *c*, and *d*, within the ascus. $\times 53$. Fig. 3. Four spores, *a*, *b*, *c*, and *d*, shot out from the ascus, showing the mucilaginous appendages on the spores. The arrangement

weeks, the three mycelia to which they had given rise produced numerous perithecia. The giant spores are evidently bisexual.

Spore-deposits obtained from perithecia produced on mycelia derived from giant spores are perfectly normal. They show no increase in giant spores over the number found in spore-deposits from perithecia produced on mycelia derived from normal spores.

V. THE SEXUALITY OF THE DWARF SPORES.

Twenty-seven pairs of dwarf spores, each pair resembling that shown in Text-fig. 4, were found in various spore-deposits; and the two spores of each pair were sown separately on sterilized horse-dung contained in small wide-mouthed bottles plugged with cotton wool. Altogether, forty of the fifty-four spores germinated: in thirteen pairs both spores germinated, while in the other fourteen pairs only one of each pair germinated.

The forty monosporous cultures derived from the forty dwarf spores were kept in the light on a table in the laboratory for upwards of two months. At the end of this time, only one of the forty cultures had produced perithecia, while the other thirty-nine had remained completely sterile. One of these sterile cultures is shown in Pl. I, Fig. 2. It is possible that the exceptional mycelium fruited parthenogenetically; but, since the spores in its perithecia were of the normal size, it is also possible that the spore from which it was derived was not a true dwarf but a normal spore of small size which had been picked up by mistake. Shear and Dodge (8) suggest the latter explanation to account for the fact that of fifty-four mycelia of *N. tetrasperma*, every one supposedly derived from a single dwarf spore, five produced fruit bodies. In picking up dwarf spores there is much less likelihood of making a mistake with *P. anserina* than with *N. tetrasperma*, because in the *Pleurage* the dwarf spores lie in pairs, whereas in the *Neurospora* they are scattered individually through the spore-deposit.

In five separate ascus spore-deposits there were in line three normal spores and two dwarf spores (cf. Text-fig. 4). The five spores of each of these deposits were isolated in succession and sown separately on sterilized horse-dung. Every one of the twenty-five spores germinated and produced a mycelium. Of these twenty-five mycelia the fifteen derived from normal

and orientation of the four spores are the same as when they were within the ascus. $\times 53$. Fig. 4. Five spores, *a*, *b*, *c*, *d*, and *e*, shot out from an ascus: *a*, *b*, and *c* are normal spores; *d* and *e* are dwarf spores. $\times 53$. Fig. 5. Three types of spores: *a*, a normal spore; *b*, a dwarf spore; *c*, a giant spore. $\times 53$. Fig. 6. Young ascus containing a fusion nucleus. $\times 100$. Fig. 7. Young ascus in which the fusion nucleus has divided to form two daughter nuclei which are side by side at the same level in the ascus. $\times 100$. Fig. 8. Young ascus with four young spores, two of which are side by side at the same level in the ascus. $\times 100$. Fig. 9. A young stained ascus which shows the two nuclei in each of the four normal spores. $\times 100$. Fig. 10. A young stained ascus which contains five spores, two of them a pair of dwarfs, and which shows the nuclei in the spores. The dwarf spores *a* and *b* each contain a single nucleus, and the normal spores, *c*, *d*, and *e*, each contain two nuclei. $\times 100$.

spores fruited about eight days after the spores were sown, while the ten derived from dwarf spores, even after the lapse of two months, remained sterile.

From about fifteen other separate ascus spore-deposits, each containing a pair of dwarf spores, one or more normal spores and one or two dwarf spores were isolated and sown on horse-dung. Here, again, the mycelia derived from the normal spores fruited readily, while the mycelia derived from the dwarf spores remained sterile.

Since, as shown by the experiments recorded above, monosporous mycelia derived from dwarf spores do not fruit, we may conclude that these mycelia are either unisexual or non-sexual.

To determine whether or not the two mycelia derived from a single pair of dwarf spores are of opposite sex, two separate sets of experiments were made: (1) the two mycelia derived from the two spores of each of thirteen pairs were planted side by side on a single mass of sterilized horse-dung in a small dish (Pl. I, Fig. 3); and (2) eight mycelia derived from eight spores, making up four pairs, were mated in all possible combinations. The production or non-production of perithecia was taken as a criterion of sex.

(1) The results of pairing the two mycelia of the thirteen pairs derived from as many pairs of dwarf spores are embodied in Table I. The two members of each pair have been designated empirically with the symbols *a* and *b*. A (+) sign indicates that perithecia were produced, while a (—) sign indicates that the mycelia remained sterile.

TABLE I.

Pairings of mycelia from the members a and b of thirteen pairs of dwarf spores.

Twelve Pairs		One Pair	
<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
<i>a</i>	— +	<i>a</i>	— —
<i>b</i>	+ —	<i>b</i>	— —

It will be seen from a glance at Table I that twelve pairs of mycelia yielded fruit-bodies, while one pair of mycelia remained sterile.

The twelve fertile pairs of mycelia derived from twelve pairs of dwarf spores fruited in the manner shown in Pl. I, Fig. 3, i.e. each pair produced a narrow band of fruit-bodies where the two mycelia came into contact with one another. Repeated pairings of the exceptional non-fruited pair of

mycelia always yielded the same result: the combination remained perfectly sterile.

(2) The results of pairing in all possible ways the eight mycelia derived from four pairs of spores are embodied in Table II. Of the four pairs of mycelia, three had been already found to fruit when paired together on horse-dung (Table I, left), while the other pair was the exceptional one (Table I, right), which when paired had remained sterile. The two mycelia of the first pair are designated with the symbols a^I and b^I , those of the second pair with the symbols a^{II} and b^{II} , and so forth. A (+) sign indicates that perithecia were produced within a month of pairing, while a (-) sign indicates that the combination of mycelia remained sterile. The mycelia which behaved alike have been placed together.

TABLE II.

All possible pairings of the mycelia from the members a and b of the pairs of dwarfs I, II, III, and IV.

	a^I	a^{II}	a^{III}	a^{IV}	b^{IV}	b^I	b^{II}	b^{III}
a^I	—	—	—	—	—	+	+	+
a^{II}	—	—	—	—	—	+	+	+
a^{III}	—	—	—	—	—	+	+	+
a^{IV}	—	—	—	—	—	+	+	+
b^{IV}	—	—	—	—	—	+	+	+
b^I	+	+	+	+	+	—	—	—
b^{II}	+	+	+	+	+	—	—	—
b^{III}	+	+	+	+	+	—	—	—

From the results embodied in Table II it will be seen that the mycelia fall into groups, those which when paired yield fruit-bodies and those which when paired remain sterile. If we assume that the production of fruit-bodies is a criterion of sexual reaction, we may conclude that the mycelia a^I , a^{II} , a^{III} , a^{IV} , and b^{IV} are of one sex, and that the spores b^I , b^{II} , and b^{III} are of opposite sex. As before, in the pairs I, II, and III, one mycelium is of one sex and the other mycelium of the opposite sex. So far as the exceptional pair No. IV is concerned, it will be noticed that both spores, namely a^{IV} and b^{IV} behave alike, and that they are therefore of one and the same sex.

From the above discussion we may conclude that, so far as individual

pairs of dwarf spores are concerned, the rule is that the two spores and the mycelia which they produce are of opposite sex, and that the two mycelia are compatible with one another in that they readily produce fruit-bodies where they meet ; and we may also conclude that, very exceptionally, the two spores of a single dwarf pair and the mycelia to which they give rise are of one and the same sex.

Since the two spores in the exceptional pair of mycelia derived from a pair of dwarf spores proved to have like sexual reactions and therefore to be of one and the same sex, and since in each young ascus there are—as we may suppose (*vide infra*)—four nuclei of one sex and four of the opposite sex, it is likely that one of the normal spores which was situated either above or below the dwarf pair was unisexual and of a sex opposite to that of the pair of dwarf spores. However, although upwards of one hundred normal spores were germinated, they all gave rise to mycelia which fruited, a fact which indicates that they were all bisexual.

Although the mycelia derived from dwarf spores can be caused to fruit by appropriate pairings in the laboratory, under natural conditions their union and fruiting must be a very rare event. There are no more than one pair of dwarfs in every 500 spores. As Professor Buller has pointed out to me, the spores, after being shot from perithecia situated on dung in fields, &c., settle on the surrounding herbage, to which they remain attached until they are eaten with the herbage by a herbivorous animal, such as a horse ; and the spores then pass down the alimentary canal of the animal concerned, so that eventually they come to be embedded in the solid excreta. It is therefore obvious that any pair of dwarf spores which has been deposited on a grass blade, &c., must have its members separated from one another in the passage down the herbivorous animal's alimentary canal, so that the chances that two dwarf spores will germinate close to one another are very small indeed.

In the hope of obtaining a race of *P. anserina* with asci containing eight spores (all of them dwarfs) from the normal species with four spores in each ascus, a series of three successive generations of *P. anserina* was grown in which the origin of each generation was a pair of mycelia derived from a pair of dwarf spores. The spore-deposits obtained from the perithecia of the first, second, and third generations, so far as could be estimated, contained no more dwarf spores than could be found in spore-deposits of equal extent taken from wild fruit-bodies. An actual count showed that a spore-deposit from wild perithecia contained 10 dwarf spores in 2,400, and that a spore-deposit from the second-generation perithecia derived from the dwarf spores contained 6 dwarf spores in 2,300.

VI. THE NUMBER OF NUCLEI IN NORMAL, GIANT, AND DWARF SPORES.

The results of investigations on the number of nuclei in the spores of *Pleurage* seem to fall in line with the findings from similar work reported for the genus *Neurospora*.

Lewis (6) investigated the eight-spored species *P. zygospora* (Speg.) Kuntze, and found that the young spores each contain *one* nucleus, although the single nucleus subsequently divides several times. Wolf (10) investigated the four-spored species *P. anserina* and found that the young spores each contain *two* nuclei. Among his material he discovered one abnormal ascus which contained three spores—one normal and two giant spores (cf. Text-fig. 1 d). The normal spore contained two nuclei and each of the giants three nuclei.

Paraffin sections of young perithecia of *P. anserina* fixed in chrom-acetic alcohol were triple stained with saffranin, gentian violet, and orange G. The nuclei of the young spores only could be made out, as in mature spores the black spore-walls obscure the cell-contents. It was found that the normal spores each contain two nuclei (Text-fig. 9), and thus Wolf's statement has been confirmed.

As we have seen, there is only one giant spore in about one thousand normal spores. On account of the rarity of giant spores, the writer was unable to find even a single one in an ascus and young enough to show the number of nuclei which it contained. It is probable that not only are there giant spores containing three nuclei each (cf. Text-fig. 1 d), as Wolf described, but that the larger giant spores observed in this investigation, which evidently replace two normal spores (cf. Text-fig. 1 c), contain four nuclei. Whether or not in *P. anserina* there are still larger giant spores which, like those found by Dodge for *N. tetrasperma*, replace three or even all four normal spores, and which contain six or eight nuclei, remains to be decided by further investigation.

Dwarf spores are not quite so rare as giant spores, as there is one dwarf spore in two hundred to three hundred normal ones; but, even so, it requires patience to find an ascus containing a pair of dwarf spores, if the spores are to be young enough to show their nuclei. In paraffin sections no asci containing young dwarf spores were found. The asci of young living perithecia were then teased out in drops of water, with the result that at length a single ascus containing three normal spores and a pair of dwarf spores was discovered. This ascus was then stained in a lactic-acid solution of cotton-blue. It could then be seen, as shown in Text-fig. 10, that the three normal spores (*c*, *d*, and *e*) each contained two nuclei, while the two dwarf spores (*a* and *b*) each contained a single nucleus.

VII. SEGREGATION OF SEX IN THE ASCUS.

Shear and Dodge (8), working with the less favourable species *N. tetrasperma*, did not succeed in obtaining both spores of any single pair of dwarf spores and then in testing the sexual reactions of the mycelia to which they gave rise. As we have seen, taking advantage of the fact that in *P. anserina* ascus spore-deposits can be found in strings (cf. Text-figs. 3 and 4), it has been possible for me to demonstrate that, as a rule, in each pair of dwarf spores one spore is of one sex and the other spore of the opposite sex.

The fact that the two spores of a dwarf pair are of opposite sex and that normal spores are bisexual indicates that, at the time of spore-formation, the ascus contains four (+) and four (−) nuclei, if we may call them so, arranged *alternately*.

Presuming that the four (+) and the four (−) nuclei in a young ascus alternate with one another, one might at first suppose that segregation of the (+) and (−) factors takes place in the third nuclear division of the ascus; but another view is possible. It so happens that the ascus of *P. anserina* is so wide that there is opportunity for the nuclei and even for the young spores (Text-fig. 8) to slip by each other. Six asci were observed in which the fusion nucleus had just completed its first division, and in all of them the two daughter nuclei were side by side at the same level (Text-fig. 7). Unfortunately, the second and third nuclear divisions have not yet been observed by me; but, presuming that they both take place transversely or obliquely as described by Dodge for *N. tetrasperma* (2), there is ample opportunity, as in that species, for non-sister nuclei to come together. If segregation of the sex factors were to take place in the first or the second or the third nuclear division, in every instance it would be possible for segregation to result in (+) and (−) nuclei finally being adjacent to one another, so that one (+) nucleus and one (−) nucleus would be included in each spore.

VIII. SUMMARY.

1. *P. anserina* is one of the Pyrenomycetes which normally has four spores in each ascus. In addition to normal spores, the asci occasionally produce giant spores or dwarf spores.

2. Usually a giant spore replaces two normal spores, but sometimes two giant spores occur in the same ascus and replace three normal spores. The dwarf spores occur in the asci only in pairs, and each pair replaces a single normal spore.

3. The spores germinate freely and the mycelium grows vigorously on sterilized horse-dung.

4. A mycelium derived from a single normal spore fruits readily. Normal spores are binucleate and bisexual.

5. A mycelium derived from a single giant spore fruits readily. Giant spores are bisexual. When a giant spore replaces two normal spores, it probably contains four nuclei.

6. A mycelium derived from a single dwarf spore remains sterile.

7. Mating experiments have shown that the mycelia derived from individual dwarf spores fall into two groups which may be called (+) and (-). When two (+) mycelia or two (-) mycelia are paired, no perithecia are produced; but, when a (+) mycelium and a (-) mycelium are paired, abundant perithecia are developed in a band where the two mycelia have come into contact. A dwarf spore is uninucleate and unisexual.

8. When two monosporous mycelia, each derived from a member of a pair of dwarf spores, are paired, they fruit readily when they come into contact with one another (one exception to this rule observed). Usually, in each pair of dwarf spores one spore is of one sex, (+), and the other is of the opposite sex, (-).

The investigation was carried out in the Botanical Laboratory of the University of Manitoba during my tenure of the Hudson's Bay Research Fellowship. It gives me much pleasure to acknowledge my indebtedness to Professor A. H. Reginald Buller for his most generous assistance. His continual suggestions and criticisms during the progress of the work have been invaluable.

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EXPLANATION OF PLATE I.

Illustrating Miss E. Silver Dowding's paper on the Sexuality of the Normal, Giant, and Dwarf Spores of *Pleurage anserina* (Ces.), Kuntze.

All figures are those of *Pleurage anserina*.

Fig. 1. On the sterilized horse-dung in the dish is a mycelium which originated from a single *normal* spore. The mycelium is about two weeks old and has fruited abundantly. The perithecia are scattered over the surface of the medium. Natural size.

Fig. 2. A similar culture, but here the mycelium originated from a single *dwarf* spore. The mycelium is more than a month old and is quite sterile. Natural size.

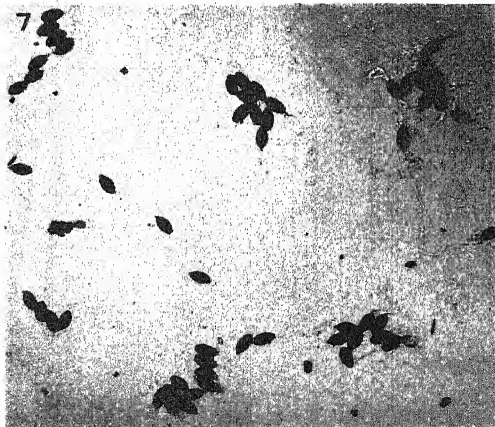
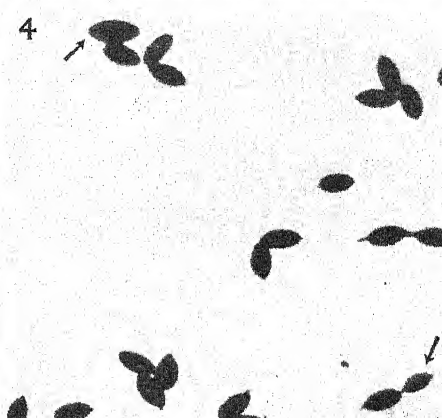
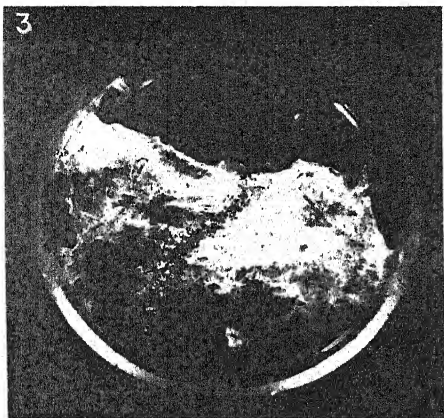
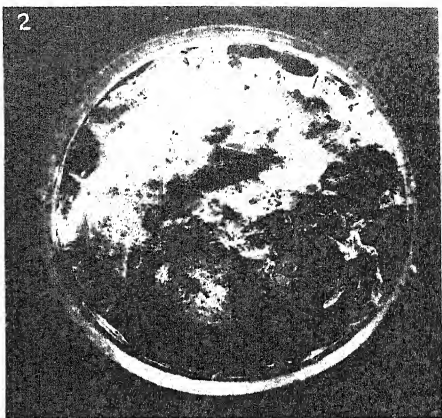
Fig. 3. A similar culture, but here two mycelia, each derived from one of the same *pair of dwarf spores*, were planted side by side. They came into contact, and where they met they have produced a band of perithecia. The two mycelia are regarded as having been of opposite sex. Natural size.

Fig. 4. A photomicrograph of a spore-deposit in which, lying among the normal spores, there happened to be one *giant* spore (left-top corner, an arrow points to it) and one *dwarf* (right-bottom corner, an arrow points to it). Magnification, about 125.

Fig. 5. A photomicrograph of a single ascus spore-deposit consisting of four normal spores. The four spores were shot obliquely on to the surface of the glass slide. The mucilaginous appendages of the spores can be faintly seen. Magnification, 60.

Fig. 6. A photomicrograph of four separate ascus spore-deposits. The sixteen spores are all of normal size. Magnification, 60.

Fig. 7. A photomicrograph of a denser spore-deposit. All the spores are normal in size. The four spores on the left, equally separated from one another, were shot from one and the same ascus, and they were connected by strings of mucilage derived from their appendages. To the right, the mucilaginous appendages of a number of spores are just visible. Magnification, 60.



Huth coll

DOWDING - PLEURAGE.

The Flowers of *Silene Saxifraga*, L.; an Inquiry into the Cause of their Day Closure and the Mechanism concerned in Effecting their Periodic Movements.

BY

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With nine Figures in the Text.

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I. INTRODUCTION.

A CERTAIN number of flowers open and close periodically. The movements in the majority of cases, take place so that the flowers are open during the day and closed during the night, but in a few cases the flowers are closed during the day and open at night. Night-flowering is generally associated with pollination by night-flying moths, the expanded petals of the flowers rendering them conspicuous when the moths are abroad.

Some night-flowering plants are well known, as for example the commonly cultivated garden plants, the tobacco, the evening primrose, and

the night-flowering stock, and the British catchflies, *Silene noctiflora*, and *S. nutans*. The blossoms of certain of the night-flowering plants have periodic movements; some of which are mentioned by Kerner and Oliver (6), among them the flowers of *S. nutans* (6, p. 154).

The periodic opening and closing of flowers attracted the attention of Linnaeus, and since his time, the problems presented by these flowers have been investigated by many botanists. These workers have been interested mainly in the day-open, night-closed flowers, and a considerable amount of information has been accumulated concerning the movements of these flowers. In the case of the night-open, day-closed flowers, however, very little is known. The significance of the day-closure, its cause and the factors which control it, have still to be ascertained.

The periodic movements of the petals of certain of the night-flowering species of *Silene* have been described and have been the subject of some experimental work. Kraus (8) in 1879 found that flowers of *S. noctiflora* remain open day and night if placed in water, or if frequently moistened, and considered that the movements depended on the contraction and expansion of the upper cells of the lamina. Lindman (10) in 1897, describing the movements of the flowers of several species and observing that closed flowers opened when placed in water, suggested that the movements were due to the different reaction of the two surfaces of the petals to changes in turgidity. And Goebel (3), figuring flowers of *S. nutans* which had opened after being forty-five minutes in water at 20°, related the movements to changes in the turgidity of the tissues.

The other records, known to the writer, of recent work on the problem of day-closure are those of de Virville and Obaton (14, 15), who in 1922 (23 and 24) described observations and experiments on the opening and closing movements of some flowers, including those of *Lychnis dioica*. They found that the daily movements in *L. dioica* depend almost entirely on temperature, though they are influenced by the humidity of the atmosphere; and that light has no action.

This paper presents a further contribution to the solution of the problems of night-flowering and day-closure. It contains the results of an inquiry into the cause of the movements of the flowers of *S. Saxifraga, L.*, and the 'mechanism' that produces them.

II. THE FLOWER INVESTIGATED.

S. Saxifraga, L., a native of the mountainous regions of southern Europe, has long been cultivated in England.¹ It is a low-growing, perennial, herbaceous plant of compact, tufted habit. The flowers are, as

¹ In Don's *Hortus Cantabrigiensis*, 13th edition, 1845, p. 301, the date of cultivation is given as 1640.

a rule, solitary, and are raised on long, stiff, erect stalks above the general level of the foliage.

The flowers are small but, when open, are made conspicuous by the whiteness of the upper (adaxial) surfaces of the petals. The closed flowers are inconspicuous, they look dingy and somewhat withered as the lower (abaxial) surfaces of the petals vary in colour from reddish brown to green.

The flowers of *S. Saxifraga*, similarly to those of some of the better known species of *Silene*, vary in character. Lalanne (9) in 1888 described *S. petraea*¹ as having flowers of two kinds, stating that sometimes the ovaries and sometimes the anthers are vestigial. Kerner and Oliver (6, p. 300) in 1895 cited *S. Saxifraga* among the Carpophyllaceous plants which are triœcious.

In the present investigation flowers of five plants were used, two of which bore only 'female' flowers, with fully-developed ovaries and vestigial stamens, while three were gynomonœcious. No flowers with vestigial ovaries and functional stamens were seen on any of the plants.

The 'female' flowers were consistently smaller than the hermaphrodite ones. The expanded limb of a petal of a 'female' flower measured approximately 4.8 mm. (average from 9 flowers), while that of an hermaphrodite flower was approximately 6.6 mm. (average from 7 flowers).

An interesting difference is found between the buds of these two kinds of flowers. The stigmas of a 'female' flower protrude beyond the petals three or four days before the bud opens. The bud in Fig. 1 was drawn four days after the first appearance of the stigmas.

The hermaphrodite flowers are markedly protandrous and the organs mature in a definite sequence, the anthers of the outer whorl of stamens, those of the inner whorl, and the stigmas, maturing in succession on three consecutive evenings. Hooker (5, p. 56) and Kerner and Oliver (6, p. 154) give the same ordered sequence of maturation of organs in the flowers of *S. nutans*, though Knuth (7, p. 162) states that these observations are questioned by Schulz.

The plants were grown in the botany garden of Bedford College, London, from seed collected in September, 1927, from a plant growing at an altitude of about 1700 m. at Gavarnie, Hautes Pyrénées. Seeds were sown in May, 1928, and the plants commenced to flower in the first week of June, 1929.

The plants produced many more 'female' flowers than hermaphrodite ones, consequently most of the observations were made on 'female' flowers.

¹ *Silene petraea*, Waldst. and Kit., apparently a form of *S. Saxifraga*.

III. THE MOVEMENTS OF THE PETALS.

The clawed, bifid petals are erect in the bud and, as a rule, their blades are convolute. The bud expands towards evening, the blade of each petal bending back until it is approximately at right angles to the claw. The flower is then open.

The flower remains open all night and very slowly closes the following morning. The petals in closing do not resume the position they occupied in the bud, but curve inwards towards the centre of the flower and gradually roll up, the curvature beginning at the apex of each lobe. While the blade is becoming thus rolled up it is slightly raised, the lifting movement taking place in the neighbourhood of the ligules. During closure the under surface of the limb becomes wrinkled and the veins appear very prominent.

The contrast presented by the appearance of a closed and an open flower is striking and is shown in Fig. 1.

The flower continues closed during the day and very gradually opens again as evening approaches. The opening process is brought about by the slow uncurling of the petal blades and their slight lowering.

The movements of the petals continue for some days and the same flower opens and closes several times. Forty-two 'female' flowers were kept under observation between July 18 and August 9. It was found that a flower opened on an average five times, the figures were:—

2 flowers opened 3 evenings					
11	„	„	4	„	
12	„	„	5	„	
14	„	„	6	„	
2	„	„	7	„	
1 flower	„	„	8	„	

The duration of the floral movements may depend on the time of change of some physiological condition brought about by pollination, but more probably the actual number of days the movements continue depends on changes in the cells due to the effect of the weather.

When the movements cease, the petals, instead of unfolding in the evening, remain curled up; they then wither and become dry.

No definite hours can be given for the opening and closing of the flowers of *S. Saxifraga* when grown in this country, they vary with the weather and, apparently, with some internal factor depending on the age of the flower. Kerner (6, p. 217) includes *S. Saxifraga* as one of the components of his 'floral' clock, and gives the time of opening of the flowers at Innsbrück, as between 6 p.m. and 7 p.m. Elsewhere, however,

he includes these flowers among the catchflies that open in the evening between 8 and 9 o'clock (6, p. 212).

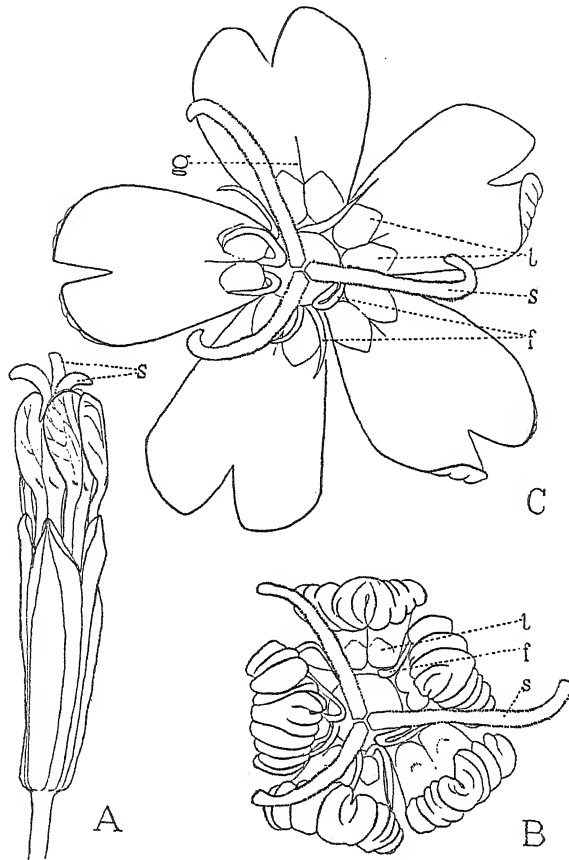


FIG. 1. A. Bud of 'female' flower with projecting stigmas, drawn four days after the first appearance of the stigmas. B and C. Hermaphrodite flower in 'female' stage, seen from above. B. 'Closed' flower, the rolled-up petals showing the ribbed abaxial surfaces. C. Open flower with expanded petals. (Same flower as B drawn next day after having been kept in water). *s.* = stigma; *f.* = filament of stamen, the anther having been shed; *l.* = 'ligule' (scale) on petal; *g.* = groove. A, B, and C. $\times 5$. Drawn with the aid of a camera lucida.

The flowers remain open longer on dry days if the soil is wet, and do not close at all on wet days.

IV. THE INFLUENCE OF ATMOSPHERIC FACTORS.

During the hours of the evening and night flowers obviously live in very different atmospheric conditions from those to which they are exposed during the day. The flowers of *S. Saxifraga* open with a decrease in light intensity and in temperature, and an increase in humidity. They

close with an increase in light intensity and in temperature, and a decrease in humidity. It would seem probable, therefore, that a change in one or more of these factors might cause the movements. Consequently, some simple experiments were made to see what effect, if any, a change in one of these three factors, light, humidity or temperature, had on the movements of the petals, when the other two factors were kept constant.

Light intensity and humidity. In one experiment three pairs of closed flowers, as similar as possible, were placed with their stalks in water and, at the temperature of the laboratory, treated as follows:—

(a) *First pair*: One was placed in the dark, and the other in the light of a north window supplemented by the light from a 100-watt lamp, passed through a water screen.

(b) *Second pair*: These were each placed in a saturated atmosphere and treated as in (a).

(c) *Third pair*: These were each placed in a dry atmosphere and treated as in (a).

The flowers behaved as follows:—

(a) Both flowers opened in approximately the same time.

(b) Both flowers opened in approximately the same time, but more quickly than the flowers in (a).

(c) Both flowers remained closed for several hours, but being left overnight were found open in the morning.

Other flowers treated similarly gave the same results.

The closed flowers supplied with water opened whether in the dark or in the light; in a saturated atmosphere or in a dry one.

The humidity of the atmosphere influenced the opening movements, flowers opening comparatively quickly in a saturated atmosphere, but only after a considerable time in a dry one.

Light intensity. The effect of an alteration in light intensity on the curvature of the petals was further tested by an experiment performed in the garden. A plant, bearing seven young flowers, was, one hot, sunny day placed in the dark, while the temperature and humidity were kept as far as possible unchanged.

The plant was covered with a light-tight box, screened from the sun. The humidity of the enclosed air was kept approximately the same as that of the outside air, by drawing (by means of a filter pump) a current of air through the box, the soil inside being covered with a piece of rubber-sheeting.

The box was removed and the plant examined every half-hour. The flowers remained tightly shut for two hours, but after that time they began to open slowly and three and a half hours later were either open, or had their petals partially unrolled, while those on the adjacent plants were still

tightly curled up. The earlier opening of the darkened flowers may have been due to the indirect effect of the absence of light, or, to an increase in the humidity of the air in the box. The temperature inside and outside the box varied 2°C ., a difference, as will be shown later, insufficient to cause movements of the petals.

The change from light to darkness has therefore not the immediate direct effect on the petals necessary to produce their movement.

Humidity of atmosphere. The retarding effect of dry air on the opening movements is more evident at a comparatively high temperature.

Three similar flowers were placed in the dark, one in a dry atmosphere at a temperature of 21°C ., while the other two were placed at a temperature of 30°C ., one in a dry atmosphere, the other in a saturated one. After an hour and a half the flowers in the saturated atmosphere at 30°C ., and that in the dry atmosphere at 21°C . had opened, but the flower in the dry atmosphere at 30°C . was still tightly closed.¹

The influence of atmosphere humidity was also tested by experiments in the garden. One sunny morning (temp. 24°C .) before the flowers had closed, the plants were watered, and one was covered with a bell-jar. The flowers in the saturated atmosphere of the bell-jar remained open while those on the adjacent plants closed.

The amount of moisture in the atmosphere is therefore an important factor in bringing about the movements of the petals. Nevertheless, the humidity of the air is not the causal factor, since closed flowers open in dry air, if they are supplied with water.

Temperature. The change from night to day is marked, normally, by a rise in temperature; it might reasonably be expected, therefore, that a change in temperature would induce the movements of the petals.

Two similar flowers were, when closed, completely covered with water. The temperature of the water was in one case raised to 37.5°C ., while in the other it was kept at 20.5°C . Both flowers opened; they were left all night and were still open next morning, when the temperatures were 34.5°C . and 20.25°C . respectively.

Similar results were obtained with closed flowers in a saturated atmosphere. It was found also that open flowers in saturated air did not close when the temperature was raised.

It would appear, therefore, that a change in temperature is not the cause that produces the movements of the petals.

These experiments were intended to be followed by others carried out under more accurately controlled conditions. However, as the results obtained showed that the movements of the petals were not directly controlled by any one of these atmospheric factors, no further experiments on these lines were made.

¹ This flower opened subsequently, when it was placed in a saturated atmosphere.

V. THE INFLUENCE OF THE WATER-CONTENT OF THE CELLS.

The influence of the water-content of the cells on the movements of the petals can be investigated, since the water-content of cells can be varied as follows:—(1) water can be abstracted from cells by immersing them in a salt solution hypertonic to their cell-sap, (2) water can be passed into cells, if they are not already 'fully turgid',¹ by placing them in a hypotonic salt solution, or in water.

When the water-content of the cells of a petal is varied by these means, movements of the petals are induced. The course of the closing and the opening movements brought about in this way can be followed in the drawings reproduced in Fig. 2.

The influence of the water-content of the cells on the position assumed by the petal was shown in three ways, (a) comparison of the time taken by petals to open when placed in a series of salt solutions of graded concentrations;² (b) comparison of the degree of curvature induced by immersion in a similar series of solutions; (c) comparison of the degree of curvature produced in a definite time, by the action of a similar series of solutions.

(a) *The influence of the concentration of the salt solution on the time taken for the petals to open.*

The rate of opening and of closing of different flowers varies considerably. It is therefore necessary in any comparative experiment to limit the number of solutions used to five, the number of petals in any one flower. If this be done the reactions of similar petals will be compared.

The petals, from a closed flower, were fixed by wax to glass blocks in such a way that their blades were free to move. One petal was placed in water and each of the others in a salt solution of different concentration, and the time required for each petal to expand was determined.

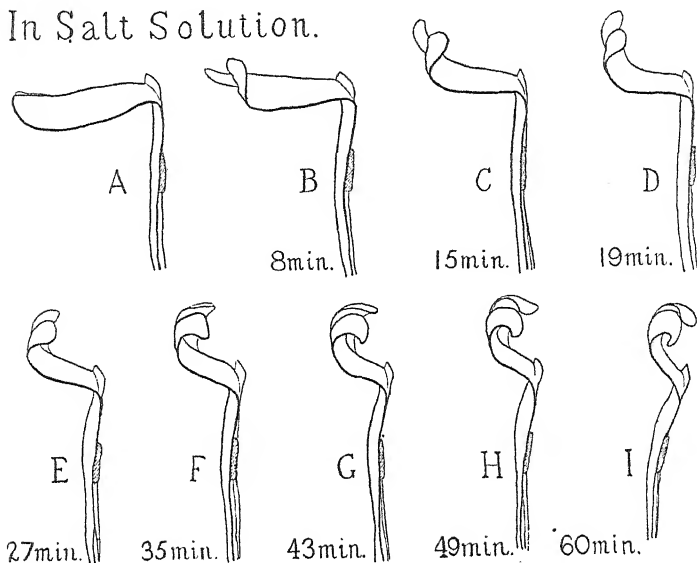
In the first experiment the petals were placed in comparatively concentrated solutions. The times of opening in the different liquids were:—

Flower.	Distilled water.	Potassium nitrate.			
		0.1 grm. N.	0.2 grm. N.	0.3 grm. N.	0.4 grm. N.
Second morning open.	17 min.	1 hr. 50 mins.	2 hrs. 50 mins.	5 hrs.	Did not open.

¹ The term 'fully turgid' is used to denote the condition of a cell when the pressure of the distended cell-wall is equal to the pressure due to the concentration of the cell-sap, and consequently, its water-content cannot be further increased.

² The salt solutions used in all the experiments were solutions of potassium nitrate.

In Salt Solution.



In Water.

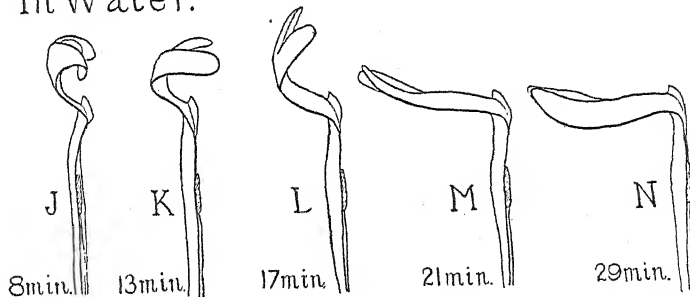


FIG. 2. 'Closing' and 'opening' of a petal, induced by abstracting water from, then adding water to its cells. Series of drawings of a petal, with a vestigial stamen attached, from a 'female' flower. A-I show the 'closing' movements induced by immersing the petal in a 0.5 gramme molecular solution of KNO_3 ; A, the form of the petal when taken from the flower; B-I, the form of the petal after immersion in the salt solution for the time stated, e.g. B, form after eight minutes in solution. I-N show the 'opening' movements induced by immersing the petal in water. I, form of petal when salt solution was replaced by water. J-N, form of petal after immersion in water for time stated, e.g. J, form after eight minutes. Vestigial anthers shaded. A-N. $\times 4$ approx. Drawn with the aid of a camera lucida.

The petals in water opened quickly, those in the salt solutions opened more slowly with increasing strength of the solution, until that in the 0.4 grm. mol. solution did not open.

Subsequent experiments were made with more dilute solutions. The results obtained are tabulated below:

TABLE I.

Time taken for the petal of a flower to open when placed in solutions of potassium nitrate.

Age of flower i. e. number of mornings open.	Distilled water.	Potassium nitrate.			
		0.025 N.	0.05 gram. N.	0.075 N.	0.1 N.
1	23 mins.	50 mins.	1 hr. 10 mins.	—	— *
4	20 "	25 "	50 mins.	2 hrs. 30 mins.	4 hrs. 45 mins.
3	45 "	1 hr. 20 mins.	—	—	— *
3	30 "	1 hr.	1 hr. 25 mins.	1 hr. 45 mins.	3 hrs. 45 mins.
1	45 "	55 mins.	1 hr. 5 mins.	1 hr. 10 mins.	1 hr. 15 mins.
2	—	55 "	1 hr. 10 mins.	1 hr. 35 mins.	2 hrs.
Average	32.6 mins.	54.2 mins.	1 hr. 8 mins.	1 hr. 45 mins.	2 hrs. 55 mins.

The times given were obtained by watching the movements of the petals and comparing the form of those in the solutions with that of the petal in water, which was thus used as a standard. The end of the opening process is difficult to determine exactly, and the times given are probably not accurate to within a few minutes.

It is evident that the time taken to open varies considerably in different flowers, the petals of older flowers opening more slowly than those of younger ones. The results show, however, that for any one flower, the time required for the opening process increases with the concentration of the solution, that is, that the time taken in opening increases when the rate of entry of water into the cells is reduced.

The influence of the rate of entry of water into the cells on the time taken for rolled-up petals to unfold is shown graphically in Fig. 3.

(b) *The influence of the concentration of the salt solution on the curvature of the petals.*

The effect of varying the amount of water in the cells on the degree of curvature of a petal was determined by experiment. Two thick longitudinal sections of a petal, each passing through the median plane of a lobe, were fastened with wax to a glass slide by the claw in such a way that the limb was free to move. The sections were then mounted in water and their form recorded. Water was then withdrawn from the cells in graduated amounts by treating the sections for a definite time¹ with 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 grm. mol. salt solutions used consecutively, and the curvature attained in each solution was recorded. Both sections reacted in a similar manner and other experiments gave the same results.

* Experiment interrupted.

¹ The sections were left at least fifteen minutes in each solution. They attained their maximum curvature in this time since sections left for a longer time did not curve any more.

The degrees of curvature attained by one of the sections¹ in the different solutions are shown in Fig. 4.

It will be seen that the curvature increased when the concentration of the salt solution was increased, that is, when more water was withdrawn

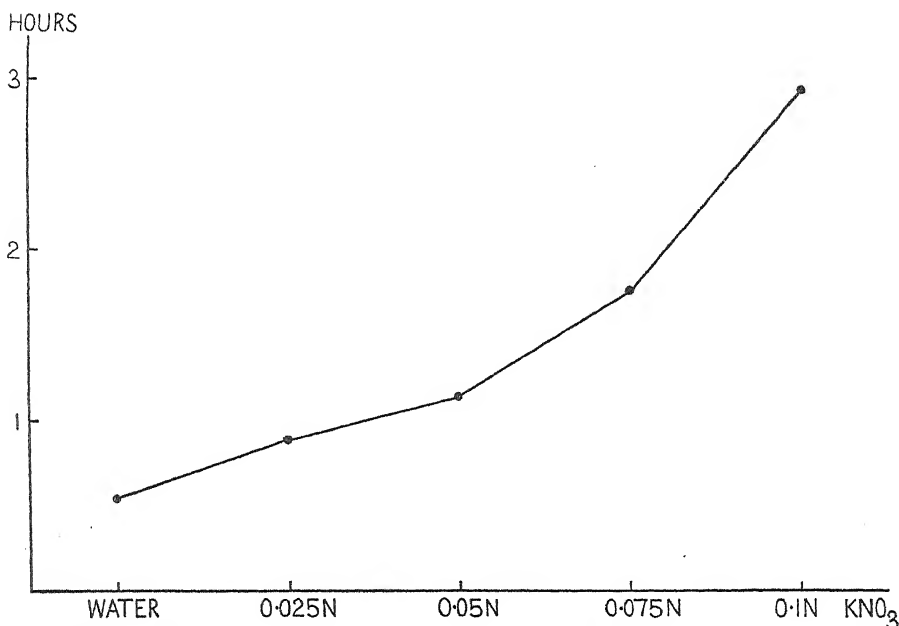


FIG. 3. Time taken for the petals of a flower to open when placed in water and in different solutions of potassium nitrate. The times plotted are the average times calculated from the results of experiments given in Table I, and the figure shows that the time of opening of a petal increases with the decrease in the rate of entry of water into the cells.

from the cells. Hence it may be deduced, that the degree of curvature of a petal varies in accordance with the amount of water in its cells.

(c) *The influence of the concentration of the salt solution on the curvature of the petals in a definite time.*

The amount of water that enters the cells in a definite time may be regulated by immersing petals in hypotonic salt solutions of graded concentrations.

The petals of a young, closed flower were placed, one in water, and the remaining four each in one of the following solutions, 0.025, 0.05, 0.075, and 0.1 gram. mol. solutions of potassium nitrate. At any time the curvature of each petal varied with the concentration of the solution in which it was immersed. The petal in water opened in thirty minutes and the difference in curvature attained in this time by the other petals is shown in Fig. 5.

¹ This section curved a little more regularly than the other section.

Hence it follows that the degree of opening attained by a closed petal varies with the amount of water that has entered its cells.

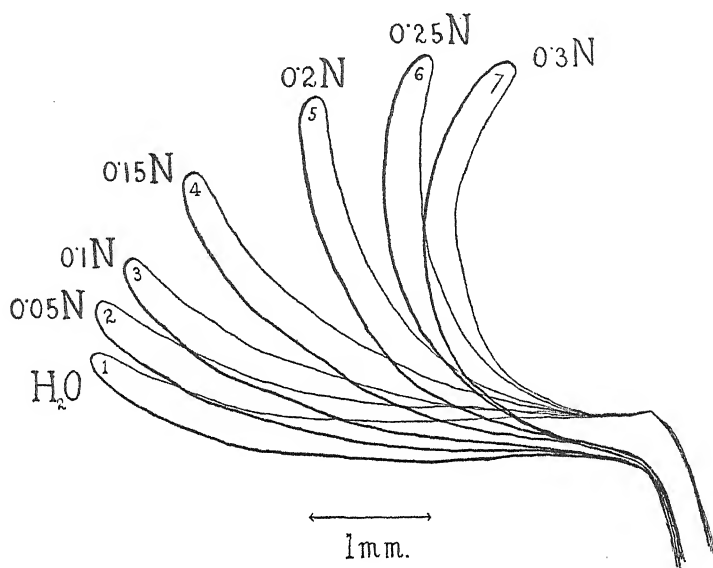


FIG. 4. The degree of curvature of a petal increases with the decrease in the water-content of its cells. The drawings, which are superimposed, of a thick longitudinal section of a petal, record the maximum curvature the section attained in water and in each of a series of solutions of KNO_3 in which it was successively immersed. The dark under-surface of the petal is drawn with the thicker line. Drawn with the aid of a Zeiss-Abbe camera lucida.

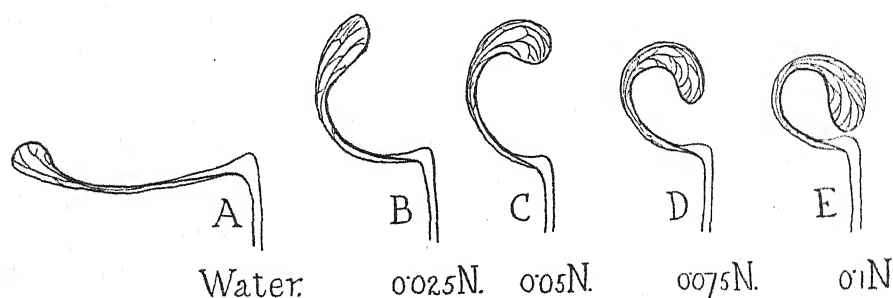


FIG. 5. The degree of curvature attained by a 'closed' petal varies in accordance with the amount of water that enters its cells. The semi-diagrammatic drawings record the various degrees of curvature (as seen in profile) of the different petals of a closed 'female' flower after immersion for thirty minutes in water and in various solutions of KNO_3 . All $\times 7$.

The movements of the petals are therefore due to turgor changes in their tissues, and the position assumed by the petals varies with the water-content of their cells.

VI. THE 'MECHANISM' OF THE MOVEMENTS.

The 'mechanism' of the movement has still to be discussed, but since it depends on the anatomical structure of the blade of the petal, this must first be described.

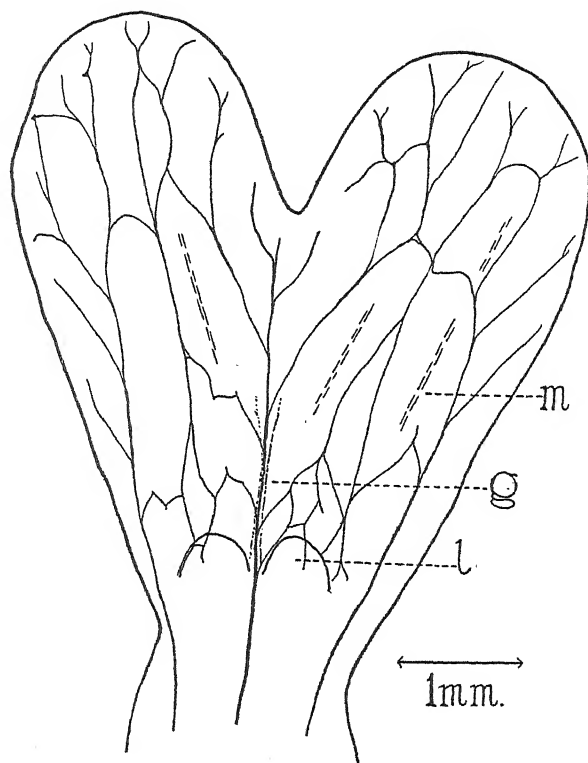


FIG. 6. *Venation of a petal of a 'female' flower.* Note the position of the endings of the vascular bundles at the edge of the blade and in the neighbourhood of the ligules. The dotted lines (*m*) indicate the plane of the long axes of the cells of the mesophyll; *l* = 'ligule' (scale); *g*. = groove on upper surface. Drawn with the aid of a Zeiss-Abbe lucida.

(a) *The anatomy of the petal.*

The anatomy of the blade is interesting and characteristic. The salient features may be described briefly under three headings:

1. *The vascular bundles.* The branching of the vascular bundles in the limb varies in detail in different petals, but the main features are the same in all the petals. Three bundles enter the limb and branch in a way similar to those in the petal drawn in Fig. 6.

The bundles are small, the xylem consisting of a few slightly lignified tracheae. A noteworthy feature of the vascular system is the position of the bundles in the mesophyll, they occur unusually near to the abaxial

surface, only a single layer of cells separating the main bundles from the epidermis (Fig. 7, A).

2. *The mesophyll.* The structure of the mesophyll is very unlike that of the foliage leaves. The cells are long, narrow, of characteristic shape

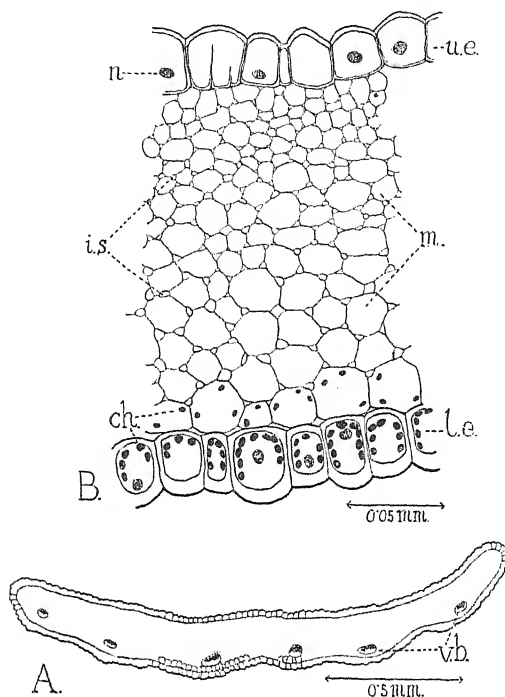


FIG. 7. *Structure of blade of petal.* A. Transverse section passing through the basal part of one lobe of a petal. Note the position of the vascular bundles (*v.b.*); B. Structure of blade of petal as seen in transverse section. Note the differences in the epidermal cells of the two surfaces of the petal and the diminution in size of the cells of the mesophyll towards the upper surfaces. No cell-contents except chloroplasts and the nuclei of the epidermal cells are shown. *u.e.* = upper epidermis; *l.e.* = lower epidermis; *ch.* = chloroplast; *n.* = nucleus; *m.* = mesophyll cell cut transversely; *i.s.* = intercellular space. A and B drawn, with the aid of a Zeiss-Abbe camera lucida, from sections of fresh material cut by means of a freezing microtome and mounted in water.

(Fig. 8), and the long axes of adjacent cells are all parallel. The direction of the long axes of the cells is shown by the dotted lines in Fig. 6. The intercellular spaces are small and form a continuous system in the blade. The cells of the mesophyll differ in size. They are largest adjacent to the lower epidermis and decrease in size towards the upper surface, as can be seen in Fig. 7, B. Chloroplasts are absent, except in the cells adjacent to the lower epidermis. These chloroplasts are small and few in number.

3. *The epidermis.* The epidermal cells are very different on the two surfaces of the petal, as shown in Fig. 7, B, and in Fig. 9.

The cells of the other type epidermis have thin cellulose walls, those at

right angles to the surface being slightly thickened locally. The cuticle covering the surface is very thin. Chloroplasts are absent from the cells, and the cell-sap is colourless.

Stomata are absent from the upper surface of the petal.

The cells of the *lower* epidermis differ in shape from those of the upper epidermis, and their form varies in different parts of the surface. Their cellulose cell-walls are comparatively thick, and the bars of thickening on the walls perpendicular to the surface are much more marked than those of the upper epidermal cells. The cuticle is thicker than that on the upper surface. Chloroplasts, conspicuous here on account of their absence in the general mesophyll, are present and starch is also found. Anthocyanin occurs in the cell-sap, the diversity of colour of the lower surfaces of the petals being due to the variation in the amount of anthocyanin present.

A few functional stomata are found in the lower epidermis, they occur mostly on the distal portion of the petal.

The noteworthy features of the anatomy of the petal are, (*a*) the position of the vascular bundles near to the lower surface, (*b*) the form and orientation of the cells of the mesophyll, (*c*) the conspicuous differences in the epidermal cells of the two surfaces.

The anatomical structure of the petal in relation to its movement will be considered later in the paper. It is necessary to examine first the effect on the form of the cells of changes in their water-content.

(*b*) *The effect produced by changes in the water-content of the cells.*

The maximum variation in the size of a cell that a change in its water-content can produce is the difference between its volumes when fully turgid and when non-turgid. A measure of this maximum difference can be obtained by comparing the area of the optical section of a cell when fully turgid, with its area when plasmolysed.

The mesophyll. Cells of the mesophyll were mounted in water, irrigated with salt solutions until plasmolysed, and the difference in size noted. In every case examined the size of the fully-turgid cell was noticeably greater than that of the plasmolysed cell. The change in size of some cells can be seen in Fig. 8. A comparison of these drawings showed that the area¹ of the optical section of cell A decreased on plasmolysis 33.6 per cent., that of cell B 33.8 per cent., that of cells D 28.4 per cent., and 25.6 per cent. respectively, giving an average decrease on plasmolysis of approximately 30 per cent. This figure indicates the degree of the maximum change in volume that occurs with the change in water-content.

The epidermis. Cells from both the upper and the lower epidermis were studied in the same way, and the changes noted.

¹ The size of each area was obtained from the camera-lucida drawings with the aid of a planimeter.

Epidermal cells do not change in size equally in all dimensions. The external wall differs from the other walls in being cuticularized and therefore inelastic, hence an epidermal cell alters in shape as well as in volume.

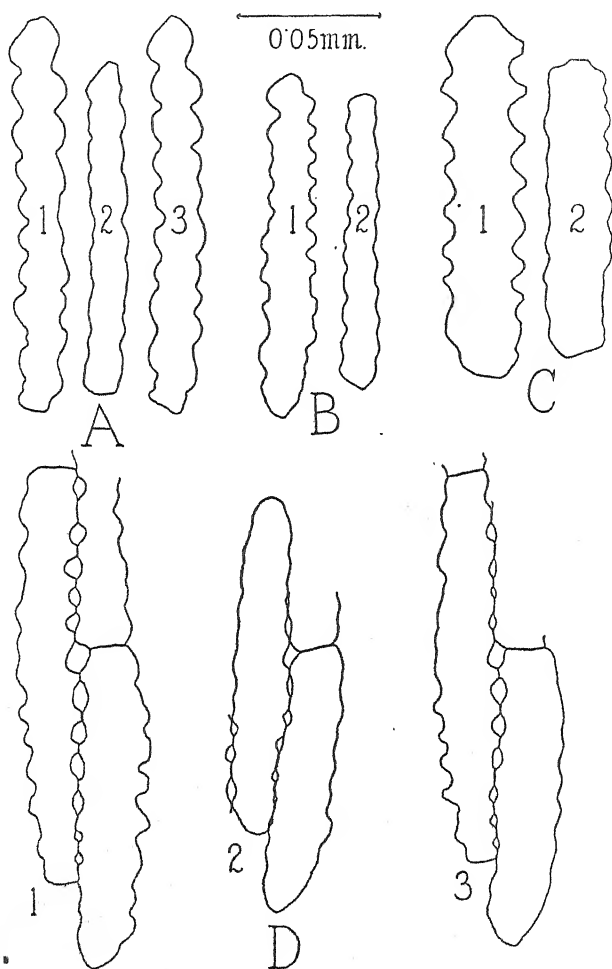


FIG. 8. *Changes in size of cells of mesophyll.* A. Cell from upper layers. 1 = fully-turgid cell from tissue mounted in water; 2 = same cell plasmolysed after irrigation of preparation with a 0.25 grm. mol. solution of KNO_3 ; 3 = same cell deplasmolysed after irrigation of preparation with water. B. Cell from upper layers. 1 = fully-turgid cell from tissue mounted in water; 2 = same cell plasmolysed after irrigation with a 0.3 grm. mol. solution of KNO_3 . C. Cell adjacent to lower epidermis. 1 = turgid cell mounted in a 0.2 grm. mol. solution of KNO_3 ; 2 = same cell plasmolysed. D. Cells from middle region. 1 = fully-turgid cells from tissue mounted in water; 2 = same cells plasmolysed after irrigation with a 0.3 grm. mol. solution of KNO_3 ; 3 = same cells deplasmolysed after irrigation with water. A-D. Optical sections of cells drawn, to same scale, with the aid of a Zeiss-Abbe camera lucida.

The cells of the upper epidermis alter considerably in passing from the fully-turgid to the non-turgid condition, the external walls become more

convex and the surface areas smaller. The area of the eight cells drawn decreased on plasmolysis 25 per cent. (Fig. 9, A and B).

The changes that take place in the cells of the lower epidermis are similar to, but not so marked as, those of the upper epidermis. The reduction in surface area on plasmolysis of the six cells drawn in Fig. 9, C and D, was approximately 14.5 per cent.

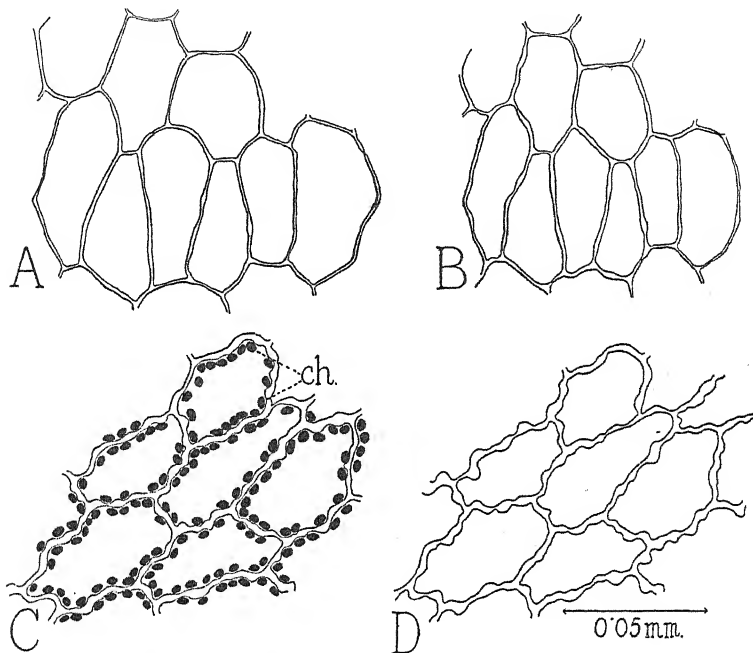


FIG. 9. *Changes in size of epidermal cells.* A and B. Cells of upper epidermis from median portion of petal. A, cells mounted in water; B, same cells plasmolysed after irrigation of preparation with 0.3 gm. mol. solution of KNO_3 . No cell-contents shown. C and D. Cells of lower epidermis from median portion of petal. C, cells mounted in water; D, same cells plasmolysed after irrigation of preparation with 0.4 gm. mol. solution of KNO_3 . *ch.* = chloroplasts in fully-turgid cells. No other cell-contents are shown. Note differences in structure of cells of the two surfaces and greater contraction on plasmolysis of the walls of the cells of the upper epidermis. Cells, seen in surface view, were drawn to same scale with the aid of a Zeiss-Abbe camera lucida.

(c) *The osmotic pressure of the cell-sap.*

The presence of chloroplasts in the lower epidermal cells is important. In the daytime carbon-assimilation goes on in these cells, sugar is formed and starch deposited.

The formation of sugar in the cells of the lower and not in those of the upper epidermis causes an increase in the osmotic pressure of the cell-sap of the former cells above that of the latter. The osmotic pressure of the cell-sap was determined approximately by the 'plasmolytic method'. On dry, sunny days the value of the osmotic pressure of the cell-sap of the upper epidermal cells was found to lie between that of a 0.25 and that of

a 0.3 grm. molecular solution of potassium nitrate, that of the lower epidermal cells between that of a 0.4 and that of a 0.5 grm. molecular solution. The difference, therefore, between the osmotic pressures of the cell-sap of the cells of the two surfaces was at least 3.5 atmospheres.

(d) *Discussion of the 'mechanism'.*

The movements of the petals of the flowers of *S. Saxifraga* have been proved to be caused by changes in the water-content of the cells. Knowledge of the details of the structure of the petal and of the behaviour of their cells now enables one to explain the way these movements are brought about.

The flower opens when the atmospheric conditions are unfavourable for transpiration and very little water, if any, is being given off from the surface of the plant. Also conditions, presumably, are favourable for the absorption of water, since the lessened evaporation from the surface soil should increase the amount of the 'available water'. Under these conditions the amount of water in the petals is progressively augmented and their tissues increase in volume until the cells become fully-turgid. The enlargement of the tissues is obvious and can be seen when a rolled-up petal is placed in water.

The flowers close when the atmospheric conditions are favourable for transpiration and water is being lost freely from the surface of the plant. At the same time water is being evaporated from the surface layers of the soil, and conditions for the absorption of water are not as good as before. Hence the action of the external conditions may cause a gradual decrease in the amount of water in the cells of the petals.

These diurnal variations in the water-content of the cells cause the periodic movements of the petals. The cells of the upper epidermis are capable of greater alteration in response to changes in their water-content than are the thicker-walled cells of the lower epidermis.

When more water passes into the closed petal than is lost from its surface the cells of the lower epidermis attain their maximum size before those of the upper epidermis. After the lower epidermal cells are fully-turgid, the distension of the cells of the upper epidermis continues, the upper surface expands and the petal slowly straightens out, the process continuing until all the cells are fully turgid, and the flower open.

When the atmospheric conditions change and become favourable for transpiration, the loss of water affects first the water-content of the cells of the upper surface. Their cuticle is thinner than that of the lower surface; and the water lost is more readily replaced in the lower than in the upper epidermal cells, since water passes into the former rather than into the latter because their osmotic pressure is greater and they are nearer to the

vascular bundles. The upper epidermal cells, therefore, lose their state of full turgidity before the cells of the lower epidermis; consequently, they decrease in size first, and, by their contraction, bring about the curving of the petal. The process continues until all the cells are no longer turgid, and the petal is closed.

The movements of the petals, therefore, are brought about mainly by the differences in the 'elasticity' of the cells of the upper and lower surfaces.

The position and distribution of the vascular bundles are, however, important in this connexion. The bundles, placed close to the lower epidermis, are very slightly, if at all, elastic, and serve as strengthening bands in the lower surface of the petal. Many of the bundles end in the lobes, but some end in the neighbourhood of the scales. Hence, when the water supply is reduced, the water-content of the cells diminishes first in the lobes and curvature begins there. The water-content of the cells gradually decreases from the apical region towards the base and the blade rolls up. Similarly, the water-content of the cells near the ligules probably diminishes at an early stage and causes the raising of the blade.

The cells of the mesophyll, expanding and contracting in response to a change in their water-content, assist the opening and closing movements of a petal.

The nature of the cell-wall is obviously important in relation to the movements of the petals, and possibly, if the loss of water continues after the cells are flaccid, drying of the cell-walls may cause the petals to become more closely rolled. The curled petals of old flowers certainly become more tightly rolled as their tissues dry.

The movements become slower, and then cease, as the flower ages, some change must therefore occur in the cells, either in the protoplasm or in the walls. This change probably takes place in the protoplast, for the movements cease and the petals become curved when the protoplasts are killed. It is possible, however, that the nature of the cell-wall also alters as the petal becomes older.

The movements are not 'growth movements' but it is possible they may continue only as long as the cells of the petal are able to increase in size. Daily measurements of marked blades showed no gradual increase in length during the period the movements persisted. However, a slight increase in length may occur, which could be determined by more precise measurements.

VII. CONCLUDING REMARKS.

The influence of external conditions on the movements of the flowers of *S. Saxifraga* may be briefly considered in the light of the information

now available concerning the cause and 'mechanism' producing the movements.

The position assumed by the blade of the petal varies with the amount of water in its cells. It follows then, that a change in any of the environmental factors that bring about an alteration in the ratio between the amount of water lost from, and the amount of water supplied to, a petal will produce a corresponding difference in its curvature.

The water-loss from the surface of plants increases with the increase in the evaporating power of the air. Renner (12), Livingston and Brown (11), and others have shown that, in certain plants, the amount of water absorbed does not increase with the increased water-loss, with the result that the water-content of the cells is diminished.¹ This condition of water-content, called by Renner 'ein Sättigungs-defizit' and by Livingston and Brown 'incipient drying',² is doubtless brought about on a dry, warm day, in the flowers, if not in the plants of *S. Saxifraga*, and causes the movements of the petals. Thus all the atmospheric factors which affect the evaporating power of the air play their part in producing the movements of the petals; and the relative importance of the different factors varies with their influence on this evaporating power.

Furthermore, temperature and light may influence the movements through their direct action on the protoplasm of the cells. An increase in temperature and in light intensity may both increase the rate of water-loss from the cells, since van Rysselberghe (13) and Delf (2) have shown that an increase in temperature causes an increase in the rate of water-loss from certain plant tissues, and F. Darwin (1) and Henderson (4), have shown that an increase in light intensity increases the rate of water-loss from the mesophyll cells of certain leaves.

Light also plays another rôle in the complex of processes that bring about the movements of the petals. The difference between the osmotic pressures of the cell-sap of the cells of the two epidermal layers is brought about by the action of light, since it is doubtless due to the sugar formed in the green cells of the lower epidermis.

Hence it follows, that the changes in all the atmospheric factors, humidity, temperature and light intensity, which mark the change from day to night conditions, produce the movements of the petals. The closure of the flowers of *S. Saxifraga* is due to the combined effect of all the atmospheric factors and not to the action of any one of them.

Other species of *Silene* are night-flowering plants. The movements of

¹ Knight (Ann. Bot., 1922) on the other hand, working in south-eastern England with *Eupatorium adenophorum* and other plants, found that the diurnal change in leaf water-content was quite small, less than 2 per cent.

² This term, which replaces Livingston's earlier term 'incipient wilting', refers to the water-content of the cell-wall, rather than to that of the cell as a whole (loc. cit., p. 314).

the petals of *S. nutans*, as described by Kerner and Oliver (6, p. 154), are very similar to those of *S. Saxifraga*. The petals expand during the evening, remain open until daybreak and then close. They become wrinkled and grooved, and are said to 'hang like five crumpled bags round the mouth of the flower'. The movements of *S. nutans* and of other species of *Silene* are like those of *S. Saxifraga*, and are probably due to a similar 'mechanism'. However, the cause and 'mechanism' of the movements of the other night-flowering species of *Silene* must be determined by experiment.

In conclusion, the possible biological significance of the day-closure of the flowers of *S. Saxifraga* may be considered. The stamens and carpels of flowers which close at night, or on a fall in temperature, are, as a rule, enclosed by the petals. It has been suggested therefore, that the closure of these flowers protects their essential organs from injury from wetting by dew or by rain, and perhaps also from the dangers of over-cooling. In the case of *S. Saxifraga*, however, the closure of the flower leaves the stamens and stigmas freely exposed. Nevertheless, the rolling-up of the petals, which only takes place when the weather is fine, may be useful biologically.

S. Saxifraga, a native of mountainous regions, grows in dry, sunny places; and its flowers, borne above the foliage on long, slender stalks, are exposed to severe atmospheric conditions. The closure of the flower decreases its transpiring surface, and hence the amount of water given off from its surface is correspondingly diminished. The reduction in water-loss renders more water available for the maintenance of the turgescence of the tissues of the stamens and carpels. These essential organs might otherwise be insufficiently supplied with water, and their tissues suffer from a water-loss from which they could not recover. The closure of the flowers of *S. Saxifraga*, in all probability therefore, prevents the cells of the stamens and carpels from being subjected to an injurious 'Sättigungs-defizit'.

VIII. SUMMARY.

1. An account is given of the movements of the petals of the flowers of *Silene Saxifraga*, L. These flowers open at night and close during the day.
2. The flowers investigated were of two kinds, hermaphrodite and 'female' flowers, the latter differing from the former in having vestigial stamens, and in their smaller size. These flowers can be easily distinguished in the bud. Both kinds of flowers were borne on some plants, but only 'female' flowers on others.
3. The petals of a bud unfold towards evening, the blades bending back until they are approximately at right angles to the claws. The flower remains open all night; in the morning the petals gradually roll up, and the flowers close. The flower remains closed until the evening, when the petals gradually unroll. On wet days the flowers remain open.

4. Flowers open and close several times. The average number of times a 'female' flower opened was five.

5. The influence of the atmospheric factors, light, temperature, and humidity on the movements was investigated. It was found that (a) a change in light intensity produces no immediate effect; (b) an alteration of temperature, in a saturated atmosphere, has no effect; (c) a saturated atmosphere hastens the unfolding of closed petals; (d) young, closed flowers always open if they are supplied with water.

6. The movements are caused by the alteration of the water-content of the cells and are therefore 'turgor movements'. Open petals close when water is taken from their cells, and closed petals open when water is added to them.

7. The relation of the amount of water in the cells to the position assumed by the petal was determined. It is found that, (a) the time a 'closed' petal takes to open is increased if the rate of entry of water into its cells is decreased; (b) the curvature of a longitudinal section of a petal increased, with the decrease in the amount of water in its cells, (c) the degree of opening of a 'closed' petal in a given time varies with the rate of entry of water into its cells.

8. The anatomy of the blade of the petal is described; the salient features are, (a) the vascular bundles lie very near to the lower surface, (b) the mesophyll cells are long and narrow, and are orientated with their long axes in the plane of the long axis of the petal; (c) the epidermal cells of the lower surface differ from those of the upper surface, their walls are thicker and they contain chloroplasts.

9. The change in volume of the cells of the mesophyll and the upper epidermis, in passing from the fully-turgid to the non-turgid state, is considerable, while the change in volume of the lower epidermal cells is comparatively small.

10. The cells of the lower epidermis have a higher osmotic pressure than those of the upper epidermis.

11. The 'mechanism' of the movements is considered, and an explanation given. The movements depend mainly on the difference in the 'elasticity' of the upper and lower surfaces of the petal.

12. The movements are probably affected by the age of the flower. The rate of movement, apparently, decreases as the flower ages and the movements cease after some days.

13. The 'closed' petals do not surround, and so protect, the stamens and carpels, nevertheless, the rolling-up of the petals may be useful biologically. The loss of water from the surface of the flower is reduced by its closure; more water, therefore, remains in its tissues and is available for the stamens and carpels, which otherwise might suffer from a deficiency of water in their cells.

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Researches on Coal-Measure Plants.

Sphenophylls from the Yorkshire and Derbyshire Coalfield.

BY

W. HEMINGWAY.

With Plates II and III and two Figures in the Text.

THE writer of the present paper had for many years the privilege of working for, and with, the late Dr. Robert Kidston of Stirling. The various reports and papers on the fossil flora of the Yorkshire coalfield, published by Kidston, were mainly based on the results of the writers collecting (7, 8, 9).

Continued collecting during the last few years has brought to light a number of specimens, some of which clear up points which were obscure to Kidston, and others which call for the institution of new species. In the following paper, the position and attachment of the sporangia of *Sphenophyllum majus*, hitherto obscure, is established, while two new species, *S. Kidstoni* and *S. Wingfieldense* are described.

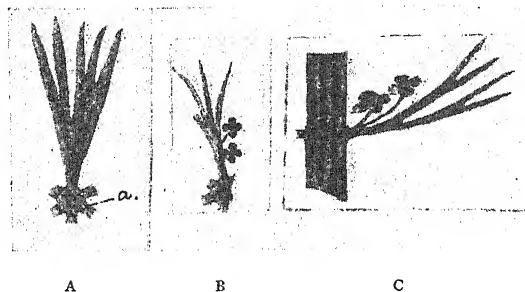
SPHENOPHYLLUM MAJUS (Bronn).

(Pl. III, Figs. 16, 16 a, and Text-fig. 1, A, B, and C).

In dealing with *S. majus* (2), the late Dr. R. Kidston described the sporangia as 'sessile on the free portion of the bract below the furcations'. (See also description and figure given by Kidston (4)). On page 129 of his Glasgow paper (3) Kidston remarks 'the sporangia are usually more or less displaced, and it is difficult to ascertain their true position on the bracts, or how many circles they formed'.

Many additional specimens have since been collected, and it was observed that the displacement of the sporangia to one or the other side of the bracts is a constant feature, this constancy is a significant fact. A careful inspection of all specimens was consequently made, and in no case could any trace of a scar be found on the limb of the bracts which might be regarded as a point of attachment of the sporangia. But in one or two instances a ring of scars is to be seen within the collarete close to the axils of the stem and bracts. Text-fig. 1, A, is a restoration showing the collarete and a leaf or bract.

The position of the sporangiophore scars is shown at point *a*. In another example a slender filament could be traced from one of the collarette scars, and bending away towards a group of sporangia on one side of the bract as illustrated in Text-fig. 1, B, sketched natural size. The lateral displacement of the sporangia is therefore seen to be due to their being attached to long flexible filaments arising within the collarette near the stem, and they were not sessile upon the limb of the bract as described



TEXT-FIG. 1. *Sphenophyllum majus*, Bronn. Restorations showing attachment of sporangia. A is an enlarged sketch of a leaf and the collarette showing the sporangiophore scars at *a*. B is a restoration of one of the verticils in specimen WH/855 showing sporangiophore and the sporangia falling to one side. C is a restoration in side view.

by Kidston. This fact brings *S. majus* more into line with other species of *Sphenophylla* in which the sporangial attachments have been definitely determined. Many fertile examples of *S. majus* have been examined, and in some cases (including one of Kidston's type specimens, WH/855) the regular order of the epidermal cells can be traced along the bract from the collarette to beyond the furcations without a break of any sort. If the sporangia had been attached to the surface of the bract, a scar of some kind, or interruption in the regularity of the surface cells would have remained to show the fact. Pl. III, Figs. 16 and 16 *a* are photographs of specimen WH/855. The specimen shows two superposed verticils of bracts and sporangia. The sporangia appear to be in two rings around the stem, but this point is not quite clear. Unfortunately the sporangiophore scars and the trace of the sporangiophore are not distinct enough to show in a small scale photograph, but they are quite clear under the microscope. They are illustrated more or less restored in Text-fig. 1, A, B, and C.

While studying the sporangia of *S. majus*, Dr. Kidston detected a second type of sporangia also in groups of fours occurring associated with those of *S. majus*. These tetrasporangia were recorded as *Sphenophyllum*, sp. (5). Kidston (6) said 'they are probably from a plant closely related to *S. majus*, but I do not think we can refer them to that species, though I know of no *Sphenophyllum* from the same horizon to which they might belong'. The writer has recently found these tetrasporangia attached to their parent plant. This is now described, and the name

Sphenophyllum kidstoni proposed for it, in honour of the late Dr. R. Kidston, who first detected it.

SPHENOPHYLLUM KIDSTONI, n.sp.

(Pl. II and III, Figs. 1-8, and 17, also Text-fig. 2).

Sphenophyllum, sp., Kidston. 'Fossil Flora of the Staffordshire coal-fields.' Trans. Roy. Soc. Ed., Vol. I, Part 1 (no. 5), pp. 131-2, Pl. X, Figs. 5, 5 A, 1914.

Sphenophyllum Kidstoni was first recorded as *Sphenophyllum*, sp., by the late Dr. Kidston in his Staffordshire paper. The earliest specimens came from Darton, near Barnsley, Yorkshire. Later, additional examples were found at Monckton Main Colliery at Royston on the same horizon. Others were found in the Brightside Rock at Brightside, near Sheffield. At the latter place the tetrasporangia occurred in great abundance associated with those of *S. majus*, and with the foliage of *S. majus* and another form of foliage which at the time was misidentified as *S. saxifragaefolium*, but which we now find is a distinct species, the *S. kidstoni* here described.

S. Kidstoni bears considerable resemblance to *S. saxifragaefolium* and hitherto appears to have been unrecognized for that reason. It differs chiefly in having normally only six leaves in a whorl and in its tetrasporangiate fructification. Pl. II, Fig. 1, gives a good idea of the general appearance of the plant. The verticils of leaves vary from 15 to 22 mm. in diameter. The leaves are deeply cleft into four to six or seven widely spreading teeth, and are joined together at the base forming a narrow collarette encircling the stem. The central split of the leaves, sometimes reaches the collarette, thereby increasing the number of leaves in a whorl. The stems vary in width from 1 to 4 mm. They are strongly ribbed as in *S. majus* (see Pl. II, Figs. 2 and 3). A few small apiculi occur on the surface of the stem but are not conspicuous. The fruiting branches do not appear to differ from ordinary foliage branches. In most specimens so far collected the sporangia are scattered on the surface of the shale, but several examples have been obtained showing the tetrasporangia *in situ* at the nodes. In three specimens, only one group of tetrasporangia could be seen at one node, but in two other examples several groups of sporangia were present. The specimens so far collected seem to indicate that the plant did not produce cones but that the sporangia occurred irregularly among the ordinary foliage. Judging from the manner in which the sporangia are scattered about on the specimens, they appear to have been easily detached. The sporangia which are *in situ* lie close to the stems and therefore appear to have been very shortly stalked—almost sessile—in the axils between the leaves and stem. The tetrasporangia consist of a central disc to which are attached four stalked pear- or fig-shaped sporangia.

When the tetrasporangia groups are seen from the underside a little scar can sometimes be observed in the centre of the disc, this undoubtedly represents the point of attachment of the sporangiophore—the short stalk which attached it to the parent plant. Occasionally a line is also present



TEXT-FIG 2. *Sphenophyllum Kidstoni*, n. sp.
Restoration showing attachment of sporangia.

along the sporangium extending from the central disc to the little notch at the outward end, this line probably represents the line of dehiscence by which the sporangium opened to shed the spores.

Pl. II, Fig. 7, shows a good example of a group of sporangia enlarged four times. The central scar on the disc can be made out, as well as a trace of the line of dehiscence. This example is seen from the underside. Spores have not yet been detected.

In *S. tenuissimum* the sporangia are 0.9 mm. broad and the central disc is reduced to a point. In *S. Kidstoni*, on the other hand, the average breadth is 1.1 mm. and the central disc is sometimes as large as the sporangium, while the sporangia of *S. majus* average 1.5 mm. broad, and the central disc is absent or nearly so.

In *S. Kidstoni* the point of attachment of the tetrasporangia to the leaf or stem has not been clearly made out. Indistinct scars may sometimes be seen on the collarette over the vein going out to the leaf, but they are too indistinct to make certain if they are the points of attachment of the sporangiophores. Scars in the middle of the sporangial disc are often quite distinct and there can be little doubt in that they represent the points where the little stalks have broken off. The surface of the sporangia is seen to consist of rows of oval cells radiating in curved lines from the stalk to the emarginate apex. In *S. majus* the cells are in about fifteen rows across the middle of the sporangium, while in *S. Kidstoni* they are in about twelve rows.

S. Kidstoni is intermediate between *S. majus* and *S. tenuissimum* both as regards its sporangia and foliage. The stem resembles that of *S. majus*, and the foliage is intermediate between the divided form of *S. majus* and the ordinary form of *S. saxifragæfolium*. Three tetrasporangiate species of *Sphenophyllum* are now known—*S. majus*, *S. tenuissimum*, and *S. Kidstoni*. In each case the sporangia appear to have been attached to a stalk or sporangiophore which arose from the collarette close to the stem. In no case has any evidence been seen of unstalked sporangia seated on the limb of the leaf as described by Kidston.

The notch observed at the ends of some of the sporangia appears to be a post mature character due to the sporangia having been split open by a ventral suture to shed the spores. Dr. Paul Bertrand has given expression to a similar view in regard to some French specimens showing this character. The same kind of notch has been observed in the sporangia of

S. majus so that this feature is of no specific importance. The number of sporangia in a group appears to be constantly four, where more appear to form a group a careful examination shows it to be due to overlapping of more than one group.

S. Kidstoni has many points of similarity to *S. majus*, but is a much smaller plant. The sporangia of *S. majus* were produced in considerable quantity at the fertile nodes and spread out almost as far as the bracts, while those of *S. Kidstoni* formed apparently only one ring close around the stem. Only one kind of leaf has been seen in *S. Kidstoni*—the kind figured—but it is possible that when we come to know the plant better, we may find that the leaves vary as in other species of *Sphenophyllum*. As observed above, the emarginate end of the sporangium is not a constant feature but appears to represent an advanced stage of development. In the young condition the sporangia are pear-shaped with rounded ends. The leaves of *S. Kidstoni* appear to have been of rather a filmy texture. In most of the specimens yet seen they have given but a faint impression on the stone, making it difficult to get contrasting photographs. The sporangia, however, give a thick layer of coal, especially in the young condition. When they have opened out to shed the spores they are more filmy.

Pl. III, Fig. 17, is one of the most distinctive examples of both leaves and tetrasporangia yet seen. The four-pointed leaves shown on this specimen are very characteristic of the species, and serve to distinguish it from *S. saxifragae-folium*. It must be remembered, however, that the number of teeth in the leaves of *S. Kidstoni* varies. Pl. II, Fig. 1, shows a variation of from four to eight teeth in the leaves. In Pl. III, Fig. 17, the veins can be distinctly made out. At the edge of the collarete there is one central vein, this immediately divides into two, and a little higher up divides again. Further divisions take place according to the number of teeth developed in the leaves.

Text-fig. 2, gives a restoration of *S. Kidstoni* in side view showing the manner in which the sporangia appear and the probable attachment to the collarete.

Notwithstanding the close resemblance of *S. Kidstoni* to *S. saxifragae-folium* it is more closely allied to *S. majus*; indeed, it might almost be looked upon as a variety of that species, a small edition with divided leaves. The fact that both species are often found together would lend support to that view. The late Dr. Kidston, however, was emphatic on the point of its being a distinct species, and the general aspect of its foliage and fructification justifies its being described as new. The writer has, therefore, great pleasure in naming it after Dr. Kidston from whom he received so many kindnesses.

Distribution of *Sphenophyllum kidstoni*.

Yorkshire coalfield:

- Hor.—Brightside Rock, Yorkian Series.
- Loc.—Brightside, near Sheffield.
- Hor.—Parkgate Coal, Yorkian Series.
- Loc.—Chappeltown, near Sheffield.
- Hor.—‘Monckton Rock’ in Barnsley Coal, Yorkian Series.
- Loc.—Monckton Main Colliery, near Barnsley.
- Hor.—Barnsley Coal, Yorkian Series.
- Loc.—Woolley Colliery, Darton, near Barnsley.

South Staffordshire coalfield:

- Hor.—Blue Measures above Brooch Coal, Yorkian Series.
- Loc.—Jubilee Pit, Sandwell Park, West Bromwich.

SPHENOPHYLLUM WINGFIELDENSE, n. sp.

(Pl. II, Figs. 9–14, Pl. III, Fig. 15.)

While studying the Fossil Plants of the Yorkshire coalfield fragments of a very small *Sphenophyllum* frequently appeared. Specimens of this plant collected from the Barnsley Coal at Darton, near Barnsley, and from the Stanley Coal at Wakefield, were recorded by Kidston (1), under the name *Sphenophyllum cf. oblongifolium*, Germar. Kidston remarks that ‘the leaves of the specimens included here under the name *Sphenophyllum oblongifolium* are much smaller than the plant figured by Germar’.

The earliest specimens were found in the shales from over the Stanley Main Coal at Manor Colliery, Wakefield, in weathered shale and were in a very fragmentary condition making identification almost hopeless. Later a few fragments were found in the upper part of the sinking at Frickley Colliery, near Pontefract. These were in shale above the Shafton Coal, Upper Yorkian. More recently the plant has been found in considerable quantity in the shales over the Kilburn Coal, Lower Yorkian (= Lanarkian Series of Kidston), at Wingfield, Derbyshire. The new specimens show it to be an undescribed species, and from its abundance at the above locality the name *Sphenophyllum Wingfieldense* is proposed for it.

The Kilburn Coal of Derbyshire is the equivalent of the Low Moor Coal of Yorkshire, and is in the Lower Yorkian Series. The Barnsley Coal and the Stanley Coal are in the Mid-Yorkian Series of the Yorkshire coalfield. The strata above the Shafton Coal are Upper Yorkian; both these divisions of the Yorkian Series—the Middle and Upper—are classed as Westphalian by Kidston. It will therefore be seen that *S. Wingfieldense* had a fairly long range in time.

S. Wingfieldense is certainly distinct from *S. oblongifolium*. The regularity of the foliage, from bifid on the smaller to linear on the larger branches, its small size and the club-shape of the bifid leaves distinguish it from the latter species, as well as from *S. angustifolium* (as figured by Renault) which it more nearly resembles. Pl. II, Fig. 9, is a photograph taken natural size and shows the plant as it usually occurs at Wingfield. Pl. II, Fig. 10, shows a more slender branch. Both specimens bear linear and bifid leaves and give a good idea of the general aspect of the plant. At *a* in Pl. II, Fig. 10, a good view of a linear leaf is seen. At *b* bifid leaves are shown. Pl. II, Fig. 11, illustrates a part verticil of leaves natural size. Pl. II, Figs. 12 and 13 show bifid leaves enlarged three times and give a good illustration of this type of foliage. It is seen that the leaves are comparatively broad for their size giving them a distinctive convex outline. Pl. II, Fig. 14 illustrates a linear leaf of the smallest type, and is seen to be fairly broad for its length. Others, as seen in Pl. II, Figs. 9, 10, and 11, are narrower and longer.

S. Wingfieldense is a very small plant with short internodes. The stems are from 1 to 4.5 mm. broad, irregularly ribbed, four to five ribs on the exposed surface, surface finely apiculate, apiculi minute, about 0.2 mm. long, and as seen at the edge of the stems are thorn-like projections with a broad base and rapidly tapering to a needle-like point. The internodes vary from 1 mm. in length in the smallest, to 17 mm. in the largest stems seen. Pl. III, Fig. 15 shows a large stem enlarged three times to show the apiculi which appear as black dots on the surface; this photograph also shows the indistinct character of the ribs, the slightly swollen nodes with the chain-like leaf scars from which the leaves have fallen. At *a* in Pl. III, Fig. 15, long linear leaves with a distinct midrib can be seen. Specimens in this condition, without any toothed leaves showing on the specimen are difficult to identify and are easily mistaken for *Annularia* or *Asterophyllites*. Many such specimens have been seen. No trifid or multifid leaves have been seen in connexion with *S. Wingfieldense*. *S. oblongifolium* as figured by Coemans and Kickx is a much larger plant with fourfid to sixfid leaves with a central cleft, a character entirely different from *S. Wingfieldense*. The outline in these two species is similar in being convex along the outer margins, otherwise these two plants have a quite different aspect. The stems of *S. Wingfieldense* are but sparsely branched as shown on Pl. II, Fig. 10. Leaves usually eight in a verticil when bifid, increasing up to sixteen when linear, joined together at the base into a narrow collarette which surrounds the stem in a funnel-like manner, as in the sheaths of an *Equisetum*. On the smaller stems the leaves are minute with an emarginate apex, the cleft deepening as the leaf grows until they become finally linear lanceolate as in *Annularia*. The average size of the bifid leaves is 4 mm. The club-shaped outline is a very

characteristic feature. The linear leaves vary from narrow to broadly lanceolate. Cones not yet identified.

S. Wingfieldense is our smallest British *Sphenophyllum*. The leaves are single-veined at the base and this continues through the whole length in the linear stage, and in the bifid stage splits evenly to send a fork centrally into each division of the leaf.

Distribution of *S. Wingfieldense*.

Hor.—Kilburn Coal. Lower Yorkian (= Lanarkian, Kidston).

Loc.—South Wingfield Colliery, Wingfield, Derbyshire.

Hor.—Barnsley Coal, Mid-Yorkian Series.

Loc.—Woolley Colliery, Darton, near Barnsley, Yorkshire.

Hor.—Stanley Main Coal, Mid-Yorkian Series.

Loc.—Mannor Colliery, Wakefield, Yorkshire.

Hor.—Shales above Shafton Coal, Upper Yorkian Series.

Loc.—Frickley Colliery, near Pontefract, Yorkshire.

I have to thank the Royal Society for a grant in aid of carrying forward my Researches on Coal-Measure Plants.

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EXPLANATION OF PLATES II AND III.

Illustrating Mr. Hemingway's paper on Researches on Coal-Measure Plants.

PLATE II.

Sphenophyllum Kidstoni, n. sp.

All the figures are taken from specimens in the writer's collection and were collected at Monckton Main Colliery, Royston, near Barnsley, in the 'Monckton Rock' (an interpolated mass of sandstone, shale, and ironstone) in the Barnsley Coal, Mid-Yorkian Series.

Fig. 1 gives a good impression of the general appearance of the plant. It shows fragments of stems, scattered verticils of leaves and tetrasporangia. Natural size. Specimen WH/2154.

Fig. 2 shows a fragment of stem natural size. This is the broadest piece of stem yet collected. WH/2158.

Fig. 3 is a fragment of stem enlarged two and a half times to illustrate the strong ribs. WH/2154.

Fig. 4 shows a verticil of leaves enlarged two and a half times to show the leaf cutting and the nervation. WH/2154.

Fig. 5 shows the veins more clearly. $\times 2.5$. WH/2155.

Fig. 6 is a bract enlarged two times showing though indistinctly several tetrasporangia *in situ*. WH/2155.

Fig. 7 shows the tetrasporangia seen on the left hand side of Fig. 8 enlarged four times.

Fig. 8 a photograph of two tetrasporangia natural size, this also shows the variation in size of the sporangia, the larger group probably having opened out to shed the spores. WH/2158.

Sphenophyllum Wingfieldense, n. sp.

All the figures are from specimens collected at Wingfield, Derbyshire. Hor.-Kilburn Coal, Lower Yorkian (= Lanarkian, Kidston).

Fig. 9 is a photograph, natural size, illustrating the general appearance of the plant, both bifid and linear leaves can be seen. WH/2322.

Fig. 10 is another example natural size. A good linear leaf can be seen at *a* and bifid leaves at *b*. WH/2323.

Fig. 11 shows two verticils of leaves one with linear and the other with bifid ones. WH/2321.

Fig. 12 part of a verticil of bifid leaves enlarged three times.

Fig. 13 two branches enlarged three times showing the convex outline of the leaves extremely well. WH/2322.

Fig. 14 a fragment of stem enlarged three times to show the convex edges of the linear leaves. The most distinct one is seen at *a*, this also shows the midrib. WH/2322.

PLATE III.

Fig. 15. Stems of *Sphenophyllum Wingfieldense* enlarged three times to show the irregular ribs and the apiculi on the surface, also the leaf scars at the nodes of the larger stem. At *a* long linear leaves with a distinct midrib can be seen. These leaves represent an advanced stage of development. WH/2324.

Fig. 16. *Sphenophyllum majus* (Bronn.). Two superposed verticils of bracts with clusters of tetrasporangia surrounding the nodes. This specimen is important as being one of the originals from which the late Dr. R. Kidston framed his description of the fructification of this plant. WH/855. Natural size.

Fig. 16 *a*. The same enlarged two times. On the uppermost verticil the sporangial scars can be distinguished (under the microscope) on the collarette, also a trace of one of the sporangiophores. Unfortunately it is not possible to show this on a small scale photograph. But a restoration is given in Text-fig. 1, A and B.

Fig. 17. *Sphenophyllum Kidstoni*. A photograph of specimen WH/2157 enlarged three times. The arrow indicates a group of sporangia seated at the base of a four-pointed leaf or bract. The emarginate character of the sporangia and the central disc are well shown. To the right of the sporangia two verticils of four-pointed leaves are shown with the veins. These leaves are very typical of the species.



1



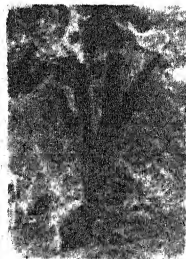
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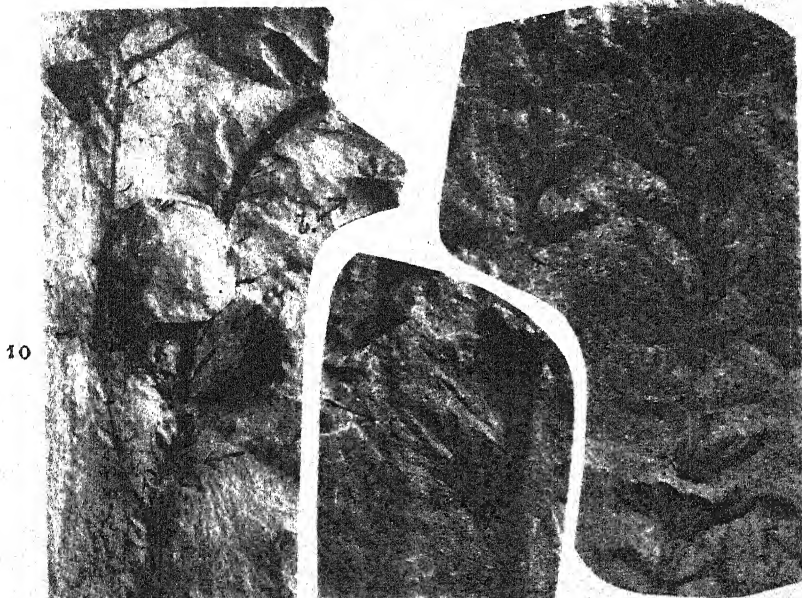
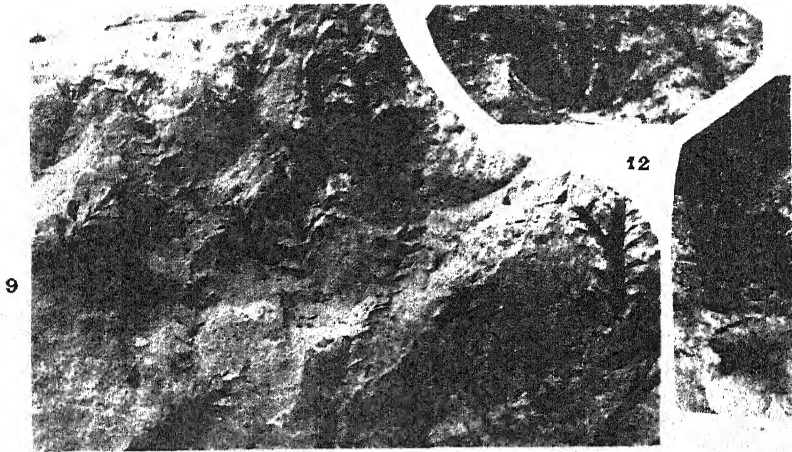
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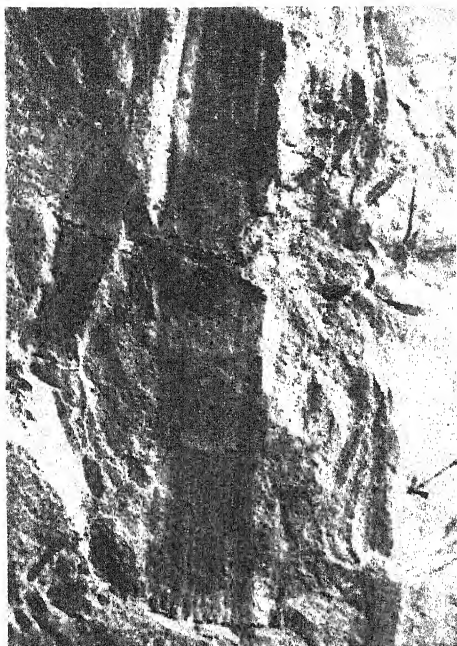
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5



15



16a



16



17



Studies in the Cytology of the Hibisceae.¹

II. The Behaviour of the Nucleus during Cell-division in the Root-tip of *Thespesia populnea* and Comparative Observations of the Phenomena in some Related Plants.

BY

W. YOUNGMAN.

With Plate IV and nine Figures in the Text.

THE nuclear phenomena about to be described have been investigated in detail in the cells of the root-tip of *Thespesia populnea*, and at the same time parallel observations have been made upon the nearly related *T. macrophylla*. Also other sets of observations have been made in the allied genera *Fugosia* (*Cienfuegosia* Cav.) and *Gossypium*. These three genera, *Thespesia* (Corr.), *Fugosia* (Juss.), and *Gossypium* (Linn.), together with the somewhat uncertain genus *Ingenhousia* (Moc; et Sess., = *Thurberia*, A. Gray), constitute according to Schumann (17), a subdivision of the capsular fruited Hibisceae which is distinguished by the possession of fused styles, and ovoid seeds. They are furthermore, as will be shown, strongly distinguished by the number of their chromosome bodies.

INTRODUCTION AND METHOD OF INVESTIGATION.

These studies in the cytology of the Hibisceae, together with a previous investigation (Youngman (20)), have been made primarily with the object of ultimately gaining some knowledge that can be applied to the understanding of the genetics of the cotton plant. The nuclear apparatus in the types of Old World cottons where the chromosome numbers are the same as in *Thespesia*, is smaller than in *T. populnea*, whilst in the larger New World cottons an impediment to investigation arises in the doubling of the number of the chromosome bodies (Denham (3)). For these reasons the method pursued has been first to make a detailed study of nuclear division in *T. populnea* as a type, and then to study the phenomena in other related plants in comparison with it. This suggested itself as a means of overcoming some of the difficulties presented by the smallness of the nuclear structure in the genus *Gossypium*.

¹ Thesis approved for the Degree of Doctor of Science in the University of London.

[Annals of Botany, Vol. XLV. No. CLXXVII. January, 1931.]

A preliminary examination of the Hibisceae from a cytological point of view showed that we have here plants whose nuclear spireme at late prophase first divides into a number of pieces which then further subdivide by transverse division. The segments which we can only say at this stage may perhaps be 'chromosomes', thus result in a larger number of 'chromosome bodies'. This property of transverse fission of the 'chromosomes' appears to be a characteristic of the Malvaceae generally. Very little attention has so far been given to this phenomenon.

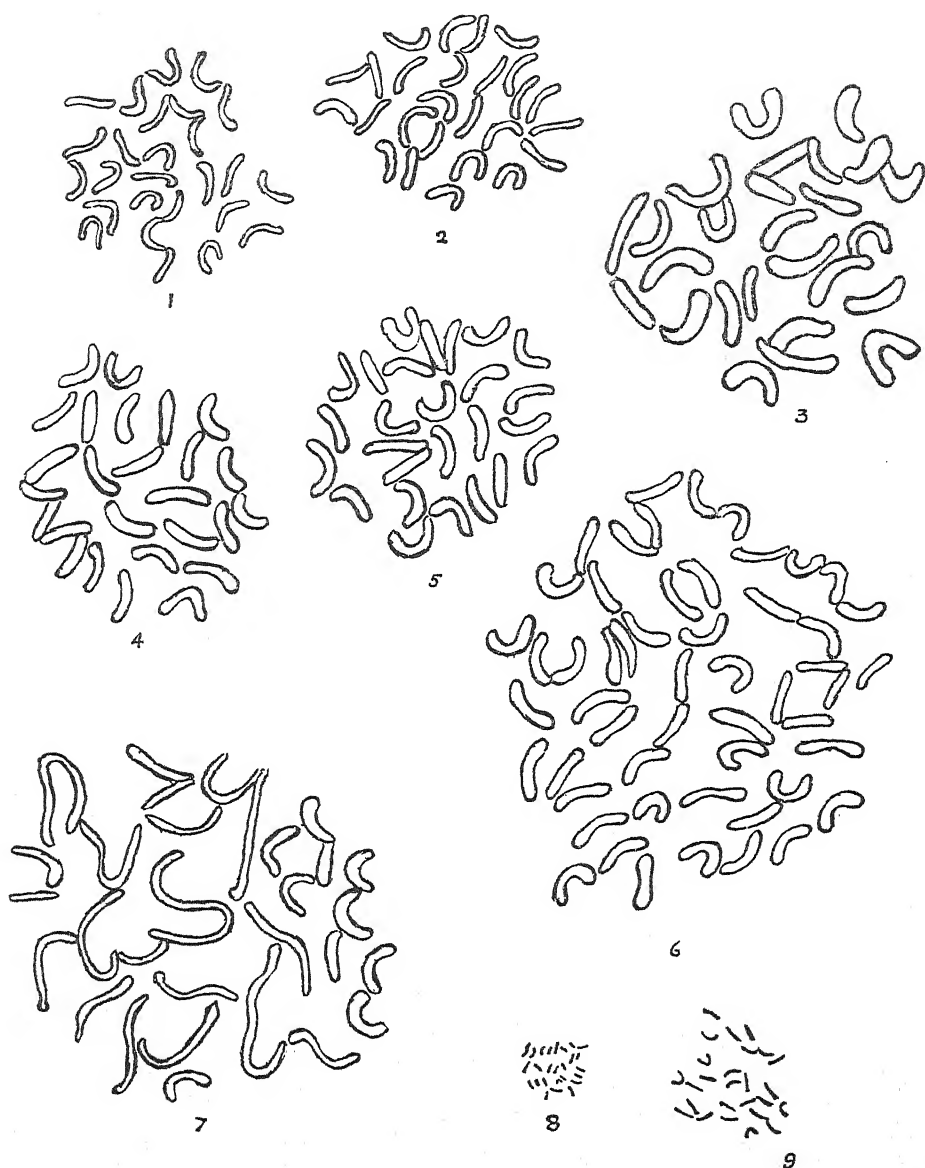
Further observation shows that amongst the Hibisceae at least two types of this transverse division of the chromosome occur, (A) one type in which a very large number of minute chromosome bodies, or spireme fragments, are found in the nucleus, this is the case in the genus *Hibiscus*, and, (B) another type, in which transverse division of the chromosome does occur, but subdivision does not proceed to anything like the extent to which it does in the first type. This is the case in the genera *Thespesia* and *Fugosia*, where the haploid chromosome bodies are twenty-six in number, and in the genus *Gossypium* where both twenty-six and fifty-two such bodies are found. The series of occurrences within the living nucleus during its division run in a cycle. The description of the nuclear phenomena in *T. populnea* will be started at the metaphase of this cycle. At this phase the chromosome bodies can be easily recognized and counted, and for this reason it forms a convenient starting point for the description. Also it enables two stages, the telophase and the prophase, the former of which may continue without interruption into the latter, to be described without a break.

THE METAPHASE.

At the completion of the prophase the segments that divide off from the spireme are chromatic, taking up deeply such stains as haematoxylin, gentian violet, and safranin. Much structure in them cannot be made out, for their size is very small. The number of the segments upon the equatorial plane is twenty-six (Text-fig. 1). The end of a segment occasionally shows a bifid appearance. The pieces finally become V-shaped, and plates of them can be found in which there are twenty-six all such shaped bodies. Signs of incomplete separation of two or more bodies are, however, to be seen on many plates, and frequently comparatively long pieces of spireme may be seen upon the equatorial plane at metaphase (Pl. IV, Fig. 1). These pieces are sometimes moniliform in outline.

By the time that the spireme has segmented into chromosome bodies the nuclear membrane has disappeared.

In equatorial plates six of the chromosome bodies can be seen at times lying somewhat separated from the other twenty (Text-figs. 1 and 2). This fact has been checked by the opinion of independent observers and



TEXT FIGS. 1-9. The chromosome bodies at metaphase in the nuclei of cells of the root-tip.
 1. *Thespesia populnea*. $\times 2,280$. 2. *Thespesia macropylla*. $\times 3,420$. 3. *Gossypium neglectum* var. *roseum*. $\times 4,560$. 4. *Gossypium herbaceum* var. *Sirwa*. $\times 4,560$. 5. *Gossypium obtusifolium* var. *sindicum*. $\times 4,560$. 6. Ashmouni (Egyptian) cotton. $\times 2,280$. 7. Marie Galante cotton. An incomplete plate. Note many of the spireme lengths have not completed subdivision. $\times 4,560$. 8. *Gossypium Stocksii*. $\times 2,280$. 9. *Fugosia Hildebrandti*. $\times 2,280$.

by making drawings of plates with the camera lucida so as to remove any possibility of the decision being influenced by the personal factor of a preconceived notion.

No distinction in size between the chromosome bodies enabling some individuals to be discriminated from others has been noticed, but the occurrence of six individuals at times apart from the rest suggests that there may be some common peculiarity in these six. By the time the metaphase stage has been reached the nucleolus has usually disappeared. A large nucleolus was characteristic of early prophase. Should any nucleolar globules exist at metaphase they are cast out into the cytoplasm, where they can sometimes be seen with a clear area around them (Pl. IV, Fig. 2). Eventually the chromosomes come to lie flat upon the equatorial plane with their two arms in that plane. By this stage the spindle fibres will have appeared. *Thespesia*, on account of the small size of the nucleus, does not furnish suitable material for the study of the method of origin of these.

Here may be examined the question as to what, regarded from the conception of the chromosomes, constitutes the difference in species between the two *Thespesias*, *populnea* and *macrophylla*. Both contain the same number of chromosome bodies in their somatic and reproductive cells, and in both species six chromosome bodies have been seen lying apart from the rest upon the equatorial plane during mitosis in somatic cells.

It is obvious from microscopic observation of chromosome plates of the two species, that the V-shaped chromosome bodies when twenty-six are upon the equatorial plane, are smaller in the case of *T. macrophylla* than in *T. populnea*. An attempt has been made to gain some idea of this difference in size by measuring under the microscope at the same magnification in each case the chromosomes of the two species. Sections of root tips prepared side by side in exactly the same solutions and by the same methods have been made, and these searched through for equatorial plates containing unbent or straight chromosomes. The method gives some idea of the size of the chromosome bodies and of comparison in size between similarly treated chromosome bodies in the two species. It is assumed that they have contracted to an equal amount in each case in the similar preparation, but of course there is no warranty for this assumption. An unbent chromosome body does occur sometimes, but it is comparatively rare, and furthermore, there is a great difficulty in deciding whether a chromosome body be straight or bent. The finding of half a dozen apparently straight chromosome bodies is a tedious task. A slightly V-shaped, or bowed chromosome, it must be remembered, would be difficult to detect from a straight one, if seen along the plane containing its two arms. Its angle would not be apparent under the microscope when thus seen, as it would be if seen at right angles to this plane. The measurements thus are liable

to error on these grounds. 'Straight' chromosome bodies, as seen under the microscope, were drawn in outline with a camera lucida at constant magnification. In this case the magnification employed was $\times 5,700$. The magnified outlines of the chromosome bodies were then measured by dividers and an accurate scale. Such measurements have been made in the case of each of the two species. For the reasons stated they furnish but a rough indication of the difference in size of the chromosome bodies of the two species.

Measurements in mm. of Straight Chromosome Bodies on the Equatorial Plate when magnified $\times 5,700$.

<i>T. populnea.</i>			<i>T. macrophylla</i>		
Length.	Width.	Calculated Volume.	Length.	Width.	Calculated Volume.
19.8	4.0	248.9	15.8	2.8	97.3
17.5	4.6	290.95	15.5	2.8	95.5
26.6	3.6	270.86	15.0	3.0	106.0
26.3	4.0	230.6	16.2	3.0	114.5
27.8	3.2	223.7	15.0	3.0	206.0
		1366.01			518.3
Average Volume 273.202			Average Volume 103.66		

From these measurements it would appear that the chromosome bodies of *T. populnea* have a volume of 2.6 times that of the chromosome bodies of *T. macrophylla*. A difference in volume of chromatin, thus, is a distinction between the two species.

A better method of comparing the size of the chromosome bodies in different varieties is to magnify plates of the different forms under the microscope so that the bodies of one type appear of the same size as those of the other, and then to compare the magnification in the two cases. For instance, the chromosome bodies of *T. macrophylla* when magnified 3,420 times are approximately equal in size to those of *T. populnea* when magnified 2,280 times, hence by this method all the chromosome bodies of *T. populnea* are some one and a half times the volume of those of *T. macrophylla*. This result is no doubt much more correct than the one above.

The chromosome bodies of the primitive cotton *Gossypium Stocksii* (Text-fig. 8) are exceedingly minute in comparison with all other cottons examined, indeed they are the smallest seen amongst the types under discussion. In their smallness they resemble those found in other subdivisions of the Hibisceae. In number they are twenty-six, in which they agree with the Asiatic cottons.

A somatic plate of twenty-six chromosome bodies of the Old World *G. neglectum* var. *roseum* (Text-fig. 3) shows the individuals about the same size as those of the fifty-two bodies in a plate of Ashmouni (Egyptian) cotton (Text-fig. 6) when the former are magnified twice as much as the

latter, and, since the number of the latter are twice as many as the former, it follows that the volume of the chromatin of Ashmouni cotton is some four times that of *G. neglectum* var. *roseum*.

The Siwa cotton of Egypt, a form with a fruit red in colour, and not green, before ripening, has its chromosome bodies as seen on a metaphase plate (Text-fig. 4) of the same number and of approximately the same size as *G. neglectum* var. *roseum*, a fact which would show its relationship with the Asiatic cottons. The external characters of the plant also indicate it to be a variety of *G. herbaceum*.

G. obtusifolium var. *sindicum* has twenty-six chromosome bodies (Text-fig. 5) of approximately the same size as the last-mentioned two Old World cottons.

The chromosome bodies in the cells of the root-tip of Ashmouni (Egyptian cotton) are slightly larger than those of *T. populnea* and double in number.

Marie Galante, a type of Sea Island cotton, frequently shows long pieces of the spireme upon the metaphase plate (Text-fig. 7). Judging by the size of individual chromosome bodies, the chromatin volume would appear to be not more than half that of Ashmouni (Egyptian) cotton. That is assuming the number of chromosome bodies to be fifty-two. It has not been possible to count them with precision.

G. hirsutum, American Upland cotton, has chromosome bodies slightly smaller than Marie Galante cotton, and less than half the size of the Ashmouni (Egyptian) cotton.

Fugosia Hildebrandti, a native of Natal, has in the nuclei of its somatic cells twenty-six small chromosome bodies (Text-fig. 9). These are larger than those of *G. Stocksii*, but smaller than those of *T. macrophylla*.

If 'the size of chromosomes may be a specific diagnostic for species whose chromosomes are, otherwise, morphologically similar and equal in number' as suggested by Ferguson (6), then on these grounds *G. Stocksii* is to be regarded as a distinct species from other Asiatic cottons, a point which also receives support from the evidence of morphology.

Further evidence in support of *G. Stocksii* being a distinct species of Old World cottons is the fact that although it can be hybridized with other Old World forms, so far the hybrid has never been found to set any seed.

All the cultivated Indian (Asiatic) cottons examined have twenty-six chromosome bodies, which are approximately equal in size. On the lines of the above suggestion this lends support to the statement of Gammie (7), who on other grounds concluded that 'From a botanical point of view it is clearly evident that we have at the most only one true species of cotton in India'. In this statement he was not including *G. Stocksii*, of which he had already stated, 'It resembles no Indian cotton'.

The New World types of cottons consistently show twice as many chromosome bodies as the Old World forms, but the volumes of chromatin vary in the different types. Twice the number of chromosome bodies does not simply imply twice the volume of the chromatin, as found by Farmer and Digby (5) to be the case in forms of *Primula Kewensis*. We are, however, comparing types of cottons which probably originated much longer ago than did these forms of the *Primulas*. Differences of environment may have acted for a much longer period of time in the case of the former than in the latter.

A superficial examination of the nuclear phenomena in the closely allied Sterculiaceae has shown that here too, as in the Hibisceae, the two types, considered with regard to the number of chromosome bodies, occur; namely, the type with a shower of minute chromosome bodies as found in the genus *Hibiscus*, and the type with fewer and larger chromosome bodies as found amongst the genera which we are now discussing. Amongst the Sterculiaceae, *Eriolaena* (Pl. IV, Fig. 3) is a good example of the first type, and *Guazuma* (Pl. IV, Fig. 4) of the second. The Malvaceae and the Sterculiaceae, without doubt, each contain some forms that have evolved from common ancestors. A fact that goes far to show descent from a common ancestor is that the highly peculiar pitted hairs derived from a peltate scale and found upon the seed coat of *Gossypium*, and forms in the stages of their evolution are found in both the Malvaceae (including the Bombacaceae) and the Sterculiaceae, and amongst no other existing orders of plants so far as we know.

In the Sterculiaceae with fewer chromosomes, Kuyper (9) has shown in *Theobroma cacao* the haploid number of these to be eight. In *Guazuma tomentosa*, a plant with near affinities to *Theobroma*, the number of chromosomes upon the equatorial plane at metaphase in cells of the root-tips is sixteen (Pl. IV, Fig. 4).

The course of division of the spireme thread in *Fugosia Hildebrandti* often shows plainly, in spite of the small structure of this thread, that the segments of spireme that arrive upon the equatorial plane undergo there a division into a penultimate number of pieces which all ultimately divide again each into two. Both the penultimate lengths and the ultimate pieces or bodies usually assume a V-shape. This makes the distinction between penultimate and ultimate portions frequently a matter of considerable difficulty unless they can be seen so as to clearly show their disparity in size, which is not always the case in a type where they are so small. For this reason, and since all the pieces do not contemporaneously divide, the decision as to what exactly is the final number of chromosome bodies upon the equatorial plane in the case of the small bodies in *Fugosia* is no easy one. It seems, however, from the study of many plates that it is twenty-six. Newly divided penultimate portions of the spireme showing their two ultimate parts

still lying in the form of a V, but perhaps separated at the angle, can frequently be seen (Text-fig. 9).

THE ANAPHASE.

Sections in a direction through or parallel to the long axis of the spindle and cutting the equatorial plane at right angles, show that soon after their arrival on the equatorial plane, many of the chromosome bodies shift their position so as to no longer lie flat in that plane, but to have their arms along a spindle fibre. The plane between the two arms of the V-shaped chromosome will thus now be at right angles to the equatorial plane. This is the beginning of the anaphase and it represents a stage at which the chromosome body has but just been caught up by a spindle fibre, before any force separating it into two longitudinal halves has come into play.

The chromosome bodies now show lacuna-like spaces, or vacuoles, at intervals along their arms. These vacuoles are non-chromatic. In stained preparations they appear as clear non-coloured areas (Pl. IV, Fig. 5). Often, in sections, one arm of the V-shaped chromosome body appears as though twisted, or corkscrew-like, in form. This is due to a vacuolated arm being cut in an oblique longitudinal direction. Such an obliquely truncated limb ends as a sharp point and is often shorter than an entire limb. Ordinarily an entire limb is cylindrical to the end. Occasionally at this stage a chromosome body can be seen cut transversely and suggesting that it consists of four longitudinally placed strands (Pl. IV, Fig. 8). This may perhaps be regarded as a precocious indication of the plane along which the next division will take place at right angles to the one actually about to happen. The vacuole-like cavities in each chromosome body coalesce so that it eventually becomes divided by vacuolation, as it were, into right and left longitudinal halves. These halves are thin in comparison with the length of the chromosome body, and, in consequence of which, they have a line-like appearance at this stage (Pl. IV, Figs. 6 and 7). The two halves next begin to separate.

Various figures are formed by the separating halves. The last point at which the separating halves actually part company is often at the two tips of the V, the two halves being pulled, as it were, at their angles by the attached spindle fibres and opening or hinging upon their tips (Pl. IV, Fig. 9). If the two longitudinal halves retain their V-shapes, then the figure formed by the separating pair of chromosome bodies just prior to actual parting will be that of a rhombus. When, as often is the case, the angle of each half is somewhat rounded, then the figure assumed will be a circle or oval. When the halves have separated they begin to move apart, the halves of each original chromosome body going towards opposite poles. At this stage the spindle is very crowded with them. They are mostly

V-shaped, but occasionally a few can be seen straight or rod-shaped, being drawn out to more or less their full length (Pl. IV, Fig. 7). Those going to either pole are usually in one horizontal plane, but at times some are nearer the pole than the others.

T. populnea is the only one of our types in which the process of longitudinal splitting of the chromosome body at anaphase has been followed in any detail. The chromosome body is larger and less crowded upon the spindle in *T. populnea* than is the case in other types.

THE TELOPHASE.

The telophase is the most difficult stage to reconstruct and interpret from a series of sections. Apart from the difficulty presented by the smallness of the structures the sequence of changes is not easy to follow and piece together into a continuous description of this phase. It appears, however, that the following is the course of events. At the poles of the spindle the chromosome bodies form a compact group. Occasionally they may be seen at the pole in a circle or coronet around the apex (Pl. IV, Figs. 10 and 11). Eventually they form an epaulette-like mass capping the spindle (Pl. IV, Fig. 13) with the two free ends of each chromosome body hanging downwards towards the equatorial plane. The free ends of a chromosome body at this stage appear somewhat swollen and more chromatic than the rest of the body. When seen from the equatorial plane the chromosome bodies thus present the aspect of many ampullae-like globules (Pl. IV, Fig. 12) hanging from the spindle apex.

At this stage a cloudy appearance becomes apparent in the area around the chromosome bodies when the preparation is stained with Heidenhain's iron-alum. It would seem that this is the result of the interaction between some secretion of the chromosome bodies and the stain. Around the chromosome mass beyond the spindle apex, on the other hand, there is a clear area in the cytoplasm (Pl. IV, Fig. 11). The cloudy appearance amongst the chromosome bodies when iron-alum has been used as a stain has been noticed by other observers. Merriman (15) in the case of *Allium* thought this to be the beginning of the formation of a new nucleolus. The nucleolus she considered to be 'only a condensation of the opaque substance to be seen in earlier stages between the chromatic bodies, being but a waste product of their activity'. Observations in *Thespesia* support the idea that the nucleolus is formed later by the excretion of substance from the chromosomes, but there is no indication that the substance causing the cloudy appearance noted above condenses to form a nucleolus. This cloudy secretion, or whatever it may be, is soon obscured by the compacting of the chromosome bodies into a highly chromatic, irregular, mass, or knot, at the pole (Pl. IV, Fig. 13). No individual

chromosome body can now be made out in this mass. Later comparatively clear spaces appear in this chromatic mass as though bubbles or vacuoles of a limpid fluid were forming therein and the outlines of chromosome bodies again become visible (Pl. IV, Figs. 14 and 15).

Although the chromosome bodies were lost to sight in the chromatic mass at the poles their subsequent reappearance indicates that they retained their individuality throughout this phase of obscurity. For the successful following of the telophase stage critical technique in the treatment of material is required. In a recent paper Zirkle (21) has shown the importance of the character of the fixing reagent when studying the chromatic structures, especially the nucleolus, the reappearance of which occurs at telophase. The staining operation, it has been found, wants exacting care. In preparations darkly coloured with Heidenhain's iron-alum much of the finer structure of the late telophase nucleus may be obscured, whilst those from which the stain has been extracted with nicety show this method to give most valuable assistance in understanding structures. Gentian violet also has been used with success, but requires to be manipulated so as to avoid anything approaching a general diffuseness. In examining the preparations under the microscope the use of a Wratten K 1 (yellow) light filter has often been found of the greatest advantage in making visible fine structures that perhaps could not otherwise be seen. Furthermore, with telophase nuclei it is most essential to visualize the plane in which they have been cut and to interpret them accordingly. From their nature telophase nuclei occur in cells that are in pairs, each cell containing one of the reorganizing nuclei. Such pairs of cells, if they be cubical ones, when cut through in a plane parallel to their sides will show the nuclei similarly cut in each case. The outlines of the nuclei will then appear as approximately equidistant from the newly-formed separating wall between the cells. This fact can often be made use of to determine the direction of the section. Nuclei at the telophase stage cut in undetermined directions frequently present insuperable difficulties to their understanding. One wants, too, to remember that sections of a nucleus are slices of a body definable by three dimensionable geometry. The interpretation of a slice as though it were the body itself has also to be guarded against.

The chromosome bodies when newly arrived at the poles and when at first compacted into a chromatic mass have no nuclear membrane around them. As soon as this mass begins to separate somewhat, so as to again allow one to see chromosome bodies, a nuclear membrane is present. The nucleus at this stage is somewhat like a double convex lens in shape, showing as an oval outline when seen in vertical sections. Within such nuclei one, or, more usually two or three, small nucleoli, and several looped chromosome bodies are to be found.

At this stage the tips of the chromosome bodies hang somewhat like

a fringe from a dense chromatic mass in which their loops are still embedded (Pl. IV, Fig. 14). This dense mass soon shows net-like openings, as apparently the diffused chromatin collects into globules in the form of nucleoli (P. IV, Fig. 15). Sometimes the chromosome bodies at this stage may show a longitudinal split-like window along their length. This fissure is very delicate and not at all easily seen, and does not appear at this stage to be general. The chromosome bodies, however, are too small to admit of a decisive statement upon this point. At this stage in the lenticular nucleus there would appear to be an actual sorting out of the looped or V-shaped chromosome bodies into pairs. A view of the nucleus from the equatorial plane (Pl. IV, Figs. 16, 17, and 18) shows numerous fine globular ends of the chromosome bodies, often in close contact with a drop of nucleolar fluid which they have apparently secreted. These globular ends of the chromosome bodies often show a grouping into four which appear as if connected by delicate cross lines. The Figures 16, 17, and 18 show more or less transverse sections at right angles to the shorter diameter of the lenticular nucleus at this stage. The stage shown in these figures resembles somewhat the description of longitudinally split chromosomes showing granules along their length, and described as occurring at telophase in some types. This, however, it seems certain is not the interpretation to be put on such stages as here depicted. In *Thespesia* a longitudinal splitting of the chromosome, as already mentioned, does not seem to generally occur at this phase.

The looped ends of the chromosome bodies lie in close contact and appear as if actually fused with one another. Amongst, and in contact with them are small nucleoli. A polar view of the convex surface of the nucleus at this stage presents an irregular network of chromatic substance (Pl. IV, Fig. 19).

The globules of chromatic fluid constituting the nucleoli have by now increased in number and volume. They may be some two, or three, and lie amongst the chromosome bodies. From this point the development of the nucleus may take one of two courses: (1) there may be a comparatively prolonged period during which the nucleus is passing to the looped spireme stage of the prophase, or, (2) there may be an almost direct passage from the telophase stage at which the looped chromosome bodies appear out of the chromatic knot, and become recognizable, to that of the looped spireme stage of the prophase.

The case where there is a series of progressive changes from telophase to prophase has been studied in considerable detail.

Much information as to the happenings during these changes can be gained not only from the study of sections of nuclei, and entire nuclei, but also by observations upon the pieces that are sometimes swept out of a nucleus by the knife in cutting. The even untoward 'misfortune' of

crushing the section when using the oil-immersion objective has furnished valuable information. One can only piece together an account from a stage found in one nucleus and another in another. It is not possible to follow the whole process itself, which would be the only way of knowing exactly what happens. The nucleus at this stage is small and it is a matter of considerable difficulty to follow events.

Nuclei showing an arrangement like a circlet of croquet hoops of fused inverted V-shape pieces within them may be found (Pl. IV, Fig. 10).

This is the first stage in the formation of a spireme loop. By the fusion of two inverted V (i. e. Λ)-shaped chromosome bodies there results an inverted W (M)-shaped piece. A transverse section across such an inverted W (or M)-shaped piece, would show a grouping of four dot-like bodies each of which represents a slice across a limb. This is the explanation of the groupings of four dots seen in sections of the nucleus at this stage and already mentioned (Pl. IV, Figs. 16, 17, and 18). Such fusions of two V-shaped chromosome bodies can often be seen. Other stages of the formation of a spireme that can be found within the nucleus at this phase are pot-hook-like pieces (\int) as shown in Pl. IV, Fig. 20, and double-hook-shaped loops (Ω) (Pl. IV, Figs. 21 and 22). Study shows these last-mentioned pieces also to result from the fusion and straightening of V-like chromosome bodies. The double-hook-shaped loop consists of four fused V-shaped bodies, its inverted W-like apex represents two, and an extra one on each side forms the long limb. The pot-hook-shaped piece is half a double-hook-shaped loop and obviously represents two fused chromosome bodies. In this way we get within the nucleus a series of forms. They are, (i) the single V-shaped chromosome body; (ii) two of them fused into a W; (iii) pot-hook-shaped pieces (\int), and (iv) double-hook-shaped loops (Ω) which finally round off at the apex into the typical inverted U (\cap)-shaped loops of spireme. It is at this stage an interesting speculation as to which of these pieces of the spireme represents a chromosome. The appearance of intermediate stages of fused lengths preliminary to the formation of the more or less continuous spireme is suggestive that transverse fission of the chromosomes has taken place. If the V-shaped units be not chromosomes, then it is obvious that we have here a case of re-fusion of the pieces of chromosomes that have at some other stage transversely segmented. If the double-hook-shaped loop be a chromosome, then it must follow, since it consists of four fused units (chromosome bodies), that the chromosomes are not all of a size, for twenty-six equal chromosome bodies cannot be equally distributed into lots of four. If the pot-hook-shaped pieces be chromosomes then they are all equal in size and are to be regarded as consisting of two units each, which units we have called chromosome bodies. At this stage, indeed, a pertinent question is—What constitutes a chromosome? For the present this speculation must be left

at this stage, until further observations on the chromosomes in the gametes be described. For this reason in the description we often avoid the term chromosome and speak preferably simply of the 'spireme length'. When we come presently to see how at prophase the continuous spireme breaks up into lengths we shall be struck by the resemblance between the method of fusion of the chromosome bodies as just described and the breaking up of the spireme. One process is indeed but the reverse of the other. During the telophase the chromosome bodies, and the loops formed by their fusion, at first occupy the cavity generally of the newly-formed nucleus. They soon form a coiled thread within the lens-shaped nucleus, but the thread is probably not at this stage a continuous one; many hoop-shaped separate pieces seem to be present. That the thread is not at this stage continuous is also the opinion of Sharpe (18) in the case of *Tradescantia*.

There is strong suggestion that the chromatic fluid of the nucleolar droplets is coming from the chromosome bodies. As the nucleolus becomes more chromatic the chromosome bodies become less so or smaller in size, and vice versa. Before the end of the telophase the chromosome loops have become highly chromatic and the nucleolar bodies smaller. Eventually the several nucleolar droplets flow together into one large globule, and this globule is so disposed that it is within the nucleus with the spireme loops peripherally situated around it. The spireme forming the loops can often be seen coming off like dendrons from the nucleolus (Pl. IV, Figs. 23 and 24). This connexion between the spireme loops and the nucleolus has been observed by several workers. It is well figured by Wager (19). On the explanation that the nucleolus is secreted by the chromosome loops this appearance can be understood. The spireme loops have now left the inside of the nuclear sphere and arranged themselves on the surface.

The spireme loops can be recognized upon the periphery of the nucleus (Pl. IV, Fig. 25). They are still compact and regularly chromatic. The looped spireme next undergoes transverse division. It is difficult at this stage to determine the length of the divisions in terms of 'chromosome bodies' as units. Almost certainly, however, the lengths are longer than the V-shaped chromosome bodies, but whether they be the two-unit pot-hook pieces, or the four-unit loops, which we saw earlier, cannot be definitely made out. All forms in a greatly elongated and irregular condition as they now become would show roughly as loops. These spireme lengths bent upon themselves into loops with delicate thread-like connexions stretching across from limb to limb, and from length to length, can be seen (Pl. IV, Fig. 26). They are at the last stage in telophasic reconstruction. The lengths are wider than when seen in earlier telophase, with a suggestion of longitudinal doubleness, and they are decidedly less

chromatic. This is the earliest stage at which the thread-like inter-connexions between them have been seen.

Sharp (18) considers that in *Tradescantia* these fine thread-like connexions are portions of the substance of limbs of the chromosomes that adhered together at telophase and stretched across from limb to limb as plastic filaments, when the limbs again opened out from the chromatic knot. This was also the view of de Litardière (10) in the case of *Polypodium*, and of Martins Mano (13) for *Solanum* and *Phaseolus*. This explanation apparently does not hold in the case of our type, for after emerging from the telophase knot no such connexions were then seen. Further, during late telophase, there has undoubtedly been a shifting of their positions and rearrangement within the nucleus by the chromosome bodies with regard to one another, and this would have destroyed the strands if they were of such an origin as suggested. A possible suggestion is that these fine threads represent portions of the limiting membrane of a vacuole of some fluid secreted by the chromosomes and expelled from them into the karyolymph. Such a vacuole would be held as a drop between the limbs of a single chromosome, or between those of neighbouring chromosomes, just as the nucleolar material is at other stages. Such vacuole membranes can often be seen in cells where a drop of a fluid borders upon a layer of different consistency, as in the case of a vacuole of fluid within the cytoplasm.

The changes that follow constitute the early prophase, and they will be described under that heading. The nucleus will by this time have passed from the lenticular shape to a spherical form. It is still surrounded by a definite nuclear membrane. There does not appear to be any marked interphase, to use the term coined by Lundegårdh for a resting period during which the chromosomes undergo a series of changes between the reconstruction period of telophase and early prophase. The telophase in *Thespesia*, it is to be noted, is itself a more prolonged change than is customary in nuclei, owing to the fusion stage of the chromosome bodies, which is not a usual one in telophasic reconstruction.

As mentioned, a second course of development through the telophase occurs. In this case cell-divisions would seem to follow one another rapidly and the outlines of the chromosomes are never lost in their passage through telophase to early prophase. The chromosome bodies early fuse together by their tips so that a continuous looped spireme results whilst the nucleus is still comparatively small and lens-shaped. The nucleolar globules flow together at one pole of this, the spireme thus forming a looped 'bouquet' around it. The nuclear membrane is early lost. By this short cut a mid-prophase stage is reached at once without the diffused attenuated condition of the chromosome at early prophase. Further mention of this will be made in describing the prophase.

Whilst these changes have been taking place at the poles of the spindle whereby a new nuclear sphere has been organized at each, the two nuclear bodies have become cut off from each other, each within its own cell, by a separating wall formed across the equator of the original cell. The formation of this wall and the behaviour of the nuclear spindle from this stage are of interest. The spindle previous to the arrival of the chromosome bodies at the poles has been distinctly fusiform in shape (Pl. IV, Fig. 6). By the time, however, that the chromosomes have reached its poles it is less fusiform and more faggot- or leaf-shaped (Pl. IV, Fig. 11). The appearance of the clear area, already mentioned, at each end of the original spindle where the chromosomes aggregate, is accompanied by the disappearance of the converging apex of the spindle cone. The fibres thus form a sheaf-like mass with the chromosomes at the ends of the sheaf. The transverse diameter across the sheaf of fibres is at first less than that of the cell within which it is. Fine granules now appear upon the equator of the sheaf of fibres midway between the two nuclei (Pl. IV, Fig. 11). These granules apparently form a plate upon the whole equatorial plane of the spindle. for the microscope can be focused upon the near circumference of the plate and upon the far circumference. The diameter of the sheaf of fibres next increases in the equatorial plane by the addition of fresh fibres on the periphery until the diameter becomes that of the cell, these fresh fibres also show granules upon their middle, so that the equatorial plate now extends right across the cell. By the already described disappearance of the fibres at the ends of the sheaf, and the increase in the equatorial diameter, a barrel-shaped cylinder of fibres results. In a cell that is rapidly growing in length the chromosome mass may somewhat leave the extremities of this cylinder. The equatorial plate of granules marks the plane along which actual cleavage in the cytoplasm takes place.

PROPHASE.

At the beginning of the prophase the spherical nucleus occupies more or less the centre of its cell. It contains a large nucleolus which stains darkly with chromatic stains like Heidenhain's iron-alum. This nucleolus is situated in the cell in the same position as would be a marble placed in a spherical glass globe. It touches the limiting membrane of the nucleus at one point just as the marble touches the glass of the globe. It is not at the centre of the nucleus. Its position is such that it would be well situated to be concerned with the intake of substance from the cell cytoplasm, which was the view of its function advanced by Montgomery (16). The period of prophase is one of increase in size of the nucleus, and this implies an addition of material from outside. Proof of the nucleolus being concerned with this function, however, does not seem to be forthcoming.

In the course of the following observations it will be seen it is rather suggested that it is concerned in some way with the diminution or increase of the quantity of chromatin in the spireme thread. Within the nucleus, occupying the space not filled by the nucleolus, in cells fixed in the usual acid-fixing solutions (Fleming's solution, Hermann's Fluid, and others), is a clear area of karyolymph, such as we might represent by water if we carried our analogy further and poured this fluid into the glass globe containing a marble representing the nucleolus. This clear zone around the nucleolus has been suggested by Zirkle (21) to be perhaps an artifact caused by the fixative. In view of the fact that sometimes there may be more than one nucleolus within a cell and that then each has its own clear zone around it, it would seem more probable that a limpid area of karyolymph around a nucleolus was rather to be explained on the lines of some repulsive force from the nucleolus as a centre acting upon particles of material, driving them centrifugally. When more than one nucleolus is found in a cell at early prophase, presumably they later fuse into one, for when the spireme is in a looped condition prior to segmenting into lengths more than one nucleolus has not been seen. Apart from the nucleolus the other chromatic substance of the nucleus is situated upon the periphery of the clear area just within the nuclear membrane. The comparison of the structure of the nucleus with a spherical glass globe containing a marble will enable one to visualize many different appearances presented by sections through nuclei at this stage. A tangential section passing through the nucleolus at right angles to the common diameter of the nucleus and nucleolus would show two concentric circular outlines, that of the nucleolus being in the centre and the other chromatic substance on the inside wall of an outer circle representing the section of the nuclear membrane. A section of the nucleus through this plane might falsely give one the idea that the nucleolus is at the centre of the nucleus. A section cutting the nucleus and nucleolus at an angle less than a right-angle to this common diameter, and looking at the cut surface of the greater half of the nuclear sphere, will show the nucleolus at the rim of an outer circle of nuclear membrane and perhaps an area of the periphery of the nucleus seen in surface view. Furthermore, it is possible to have a section showing a circle of the circumference of the nucleus without showing the nucleolus at all. These simple conceptions require to be remembered in interpreting the appearances of sections of the nucleus and its contents at early prophase. As simple as they are, attention is often not paid to them in explaining sections at this stage, and a wrong idea of the nucleus is thus given.

The chromatic material around the periphery of the nuclear sphere, within the nuclear membrane, is the greatly attenuated spireme thread, or chromosome portions of it. This attenuated form of chromosome portions is no doubt in part due to their elongation with the generally enlarging area

of the nuclear sphere that is taking place at this stage. The chromatin in them is distributed irregularly into intermittent patches as a result apparently of the attenuation or elongation of the chromosome portions upon the inner surface of the enlarging nuclear sphere, there not being enough chromatin as it were to occupy evenly the whole of the expanding lengths. As McClung (14) has put it: 'A newly formed cell is, in most cases, one of two nearly equal cells. It contains, part by part, half of the parent unit, but each of these is half the original size. Before another division can occur, some approximate restoration to normal proportions of all portions of the cell must take place. Material from outside must be brought in. . . . The chromatin while in the growth changes has disposed itself upon the nuclear membrane so as to offer the greatest possible surface at this osmotic barrier.' The prophase is essentially a stage of the absorption of increment-producing substance, which restores the size of the chromosomes to that which they possessed prior to having undergone division.

Bearing in mind the description given of the arrangement of the parts of the nucleus it will be obvious that to study the spireme portions at this stage we want to view areas of the surface of the nuclear sphere. In very thin sections of cells some will perhaps contain a slice cut tangentially from off the nuclear sphere. In such slices (Pl. IV, Fig. 28) looped arrangements of skeleton-like portions of the spireme can be seen. They resemble the chromosomes of *Tradescantia* at the same stage, as figured by Sharp (18).

The width of these pieces of spireme is much greater than seen at any previous stage. They are greatly vacuolated and have a most irregular appearance, often consisting of little more than the walls of vacuoles showing like rings arranged in a chain. Presumably the spireme length is secreting some fluid and the clear spaces or vacuoles are the places of formation of droplets. The secretion is not chromatic when treated with the usual stains. Chromatic nucleolar material in the form of globules is scanty, or absent from the nucleus, at this stage.

Sometimes the spireme length appears as a corkscrew-like or spiral line (Pl. IV, Fig. 28, left side) rather than as a series of contiguous rings. This is considered by Sharp (18) to be the next stage in the behaviour of the spireme and is what he figures as 'chromosomes in single thread stage' and which he considers to be a regular condition following the reticulate, or ringed, phase just described. The same condition in *Allium* is figured and described by Bonnevie (1).

Following the corkscrew-like condition of the spireme thread is a stage in which it passes through a very jumbled condition (Pl. IV, Fig. 29) upon the surface of the nuclear sphere. At this stage the corkscrew-like curves are no longer seen. It comes out of this tangle into a more regularly

disposed thread. Throughout these last stages the spireme is becoming more chromatic as well as more regular, both processes being perhaps the result of a condensation of the thread. The spireme now becomes folded into characteristic loops upon the surface of the nucleus, and at this stage a distinct longitudinal split dividing the thread into two parallel portions can often be seen (Pl. IV, Fig. 30). This split again becomes obscured at the end of the prophase. Occasionally a length of spireme shows a ladder-like arrangement with cavities square in shape. In such a case there appears to be a chromatic dot where delicate transverse-line-like staves of chromatic material join the side on either hand. This stage is possibly a somewhat precocious early prophase manifestation of a split, as the width of the thread in such a stage is comparatively very great. The thread in the later phase often shows a metameric series of chromatic granules which are obviously balanced on the right and left longitudinal thread formed by the split (Pl. IV, Fig. 30). As already stated, the thread is condensing, and it has become continuous by the separate lengths coming in contact at their ends.

A continuous spireme thus now lies upon the periphery of the nuclear sphere. It may be compared to a thread wound upon the nucleus, but not quite in the way that one would wind a thread around a bobbin or ball, for instance. Its length at intervals turns back in a loop so as to run parallel to its former direction without having made a complete circuit of the nucleus. The loops of the spireme thus formed generally do not touch one another; the crossing of one portion by another is unusual. The whole spireme thus is never fully extended, but consists of a series of U-shaped loops (Pl. IV, Fig. 31).

There is at this stage a polar disposition of the nucleus with regard to the looped spireme. The loops have their apices all directed towards one pole whilst at the opposite pole is situated a nucleolus. The spireme strands in many cases seem to be in intimate contact with the nucleolus. This condition has previously been seen at telophase and was accounted for there as the result of the nucleolus being secreted by the spireme strands.

The spireme is apparently folded into some six to eight loops upon the periphery of the nuclear sphere. It is a difficult matter to come to a decision as to the number of loops, because they may show subsidiary folds. (Sometimes about this stage the spireme shows a distinctly moniliform appearance which may persist up to the metaphase (Pl. IV, Fig. 27). It is possible that this moniliform condition is characteristic of loops of spireme that have passed direct from the looped stage at telophase to this second looped condition at late prophase.) It is not easy to get a view in which all the loops of the spireme can be seen uncut and at one time. When one sees six loops that does not preclude the presence of another one or two which do not project so far over the nucleus as the others, and

consequently are not seen. A polar aspect of the nucleus looking along an inclined axis upon the tips of the folds of the spireme is the one in which the most, or all, of the folds can be seen at once. This folded spireme next divides transversely into a number of pieces or lengths. Frequently an inverted U-shaped loop breaks off by a fission across each limb in a region towards the nucleolar pole. The number of pieces into which the spireme at first segments on the periphery of the nucleus is some eight (Pl. IV, Fig. 33). The two free ends of the Ω -shaped pieces may begin to bend upwards so as often to give the loop a mitre-shaped, or double hooked appearance Ω . At this stage a fission of the mitre-shaped piece into two occurs, by a transverse splitting at the apex. In this way a number of pot-hook-like pieces of the shape \int results. These pieces of the spireme next come to lie upon the equatorial plane. By the time that the pot-hook-like pieces have reached the equatorial plane they themselves have often started to divide transversely into two segments each. Sometimes longer pieces of the spireme than these pot-hooks are to be found dividing upon the equatorial plane (Pl. IV, Fig. 1).

A study of the spireme length shows that fission into segments occurs at places which sometimes can be seen just prior to actual division. The separation would seem to be effected by a process of bending of the segment upon itself, this bending causing the two ends of two segments to move apart at the dividing line.

The whole spireme becomes, in the way described, transversely divided up into a number of pot-hook-like pieces. The division, however, of all the spireme loops is not always synchronous and frequently comparatively long pieces, double-hooked pieces, pot-hook-like pieces, and smaller lengths can be found in the same nucleus. The smaller lengths are formed by each pot-hook-shaped piece dividing transversely into two and these are the ultimate pieces into which the spireme divides. In both *Thespesia populnea* and *T. macrophylla* they are twenty-six in number. These twenty-six chromosome bodies, as we may now call them, have thus been derived from thirteen penultimate pot-hook-like portions of the spireme. Owing to the non-synchronous division of the spireme thread a stage with thirteen similar pot-hook-like pieces is not found. Actually, however, many, if not thirteen, pieces, each showing division into two, often are to be found upon an equatorial plate. Pieces too are frequently to be seen lying in pairs with their tips at one end near each other, after the manner of homologous chromosomes.

The thirteen pieces are herein spoken of as the 'chromosome portions' and the twenty-six ultimate particles as the 'chromosome bodies'. The discussion as to which of these represents a chromosome will be left until we have considered the formation of such bodies as are seen in a study of the male sexual cells.

SUMMARY.

1. The nuclear phenomena during the division of somatic cells in the genus *Thespesia* are investigated and described with comparative notes upon the process in the allied genera *Fugosia* and *Gossypium*.

2. In the Hibisceae the nuclear spireme at late prophase first divides into a number of pieces which then further subdivide by transverse division. This transverse fission of the 'chromosomes' into 'chromosome bodies' is characteristic of the Malvaceae generally.

3. In the Hibisceae there are two types of this fission one in which a shower of minute chromosome bodies results, as is the case in the genus *Hibiscus*, and another type in which the subdivision results in fewer numbers of chromosome bodies. This latter is found in the allied genera *Thespesia*, *Fugosia*, and *Gossypium*. In the first two genera twenty-six chromosome bodies have been found, and in *Gossypium* types with both twenty-six and fifty-two occur.

4. At metaphase in equatorial plates of *T. populnea* and *T. macrophylla* six of the chromosome bodies sometimes can be seen lying apart from the other twenty.

5. The chromatin volumes of the nuclei at metaphase of *T. populnea* and *T. macrophylla* are compared by methods of direct and comparative measurement. A conception of species difference is a difference in the volume of the chromatin of the nucleus.

6. Comparisons are made between numbers of chromosome bodies and the chromatin volumes of the nuclei of types of the genera *Thespesia*, *Gossypium*, and *Fugosia*. The same two types of nuclear phenomena considered with regard to the number of chromosome bodies occur in both the Hibisceae, and the Sterculiaceae.

Amongst the Sterculiaceae, *Eriolaena* is an example of a form with a shower of minute chromosome bodies. Amongst forms with a smaller number *Theobroma* has been shown to have eight haploid bodies, and *Guazuma* has sixteen bodies upon the metaphase plate of somatic cells.

7. The longitudinal splitting of the chromosome bodies at anaphase begins by the appearance of vacuole-like areas along them, which areas eventually increase and coalesce dividing the chromosome body into right and left halves. The longitudinal halves travel apart to the opposite poles of the spindle as V-shaped bodies.

8. The chromosome bodies compact themselves into a chromatic knot at the spindle poles. Later clear spaces appear in this mass and the outlines of chromosome bodies again become visible. At this stage a nuclear membrane appears around the nucleus, and small nucleoli within it.

9. At the stage of reappearance of the chromosome bodies they at first hang with their free ends projecting from the chromatic mass in which

their loops are aggregated. Globules of chromatic fluid apparently secreted by the chromosome bodies lie amongst them. These are the newly appearing nucleoli.

One of two courses may follow :

- (i) There may be a comparatively prolonged period with a series of progressive change during which the nucleus is passing to the looped spireme stage of the following prophase;
- or, (ii) there may be an almost direct passage from the telophase to the prophase.

The case where there is a prolonged period of a series of progressive changes has been studied in detail.

When the chromosome bodies have reappeared at telophase they undergo a reconstruction into a more or less continuous spireme. In this process two inverted V-shaped bodies fuse into an inverted W form, one V-piece more joins on to each side limb of this so that four pieces now form a double-hook-shaped loop Ω . The angles in such a loop straighten out into a more regularly curved loop. Sometimes pot-hook-shaped pieces are found, these represent half a double-hook-shaped loop each, and consist of two fused chromosome bodies.

It is suggested that we have here a case of the refusion of pieces of chromosomes that at some other stage have transversely segmented.

The last stage in telophasic reconstruction shows the spireme loops much wider and less chromatic than when previously seen. Delicate thread-like connexions stretched across from limb to limb and from loop to loop.

10. By early prophase the nucleus will have become spherical in form. Where cell-divisions follow one another rapidly it seems the outlines of the chromosomes are never lost in their passage through telophase to early prophase. The chromosome bodies early fuse by their tips forming a continuous looped spireme arranged as a 'bouquet' around a nucleolus. By this short cut a mid-prophase stage is reached without the diffused attenuated condition of the chromosomes at early prophase.

By early prophase a cell plate will have formed between the two sister nuclei.

11. The arrangement of the constituent parts of the nucleus at early prophase is described. The nucleolus is situated near the nuclear membrane at one point, other chromatic material at the periphery within the nuclear membrane and the space within the membrane not occupied by the chromatic bodies (nucleolus and spireme) is filled with clear karyolymph.

12. In thin tangential slices of the periphery of the nucleus at early prophase looped skeleton-like portions of the spireme may be found.

The width of these pieces is greater than at any previous stage and

they have an irregular appearance, often consisting of little more than the walls of vacuoles arranged in a chain. The spireme length would seem to be secreting some fluid which is not chromatic with the usual stains. The vacuolated condition of the spireme is followed by a corkscrew-like thread stage, and then by a jumbled up condition upon the nuclear surface, finally the spireme emerges from this tangle as a more regularly disposed thread which becomes more chromatic and thicker. The spireme next shows folds or loops upon the surface of the nucleus. A distinct longitudinal split dividing the spireme into parallel halves can at this stage be found. This split becomes obscured again at the end of prophase. By the separate lengths coming in contact at their ends a continuous looped spireme lying upon the periphery of the nucleus results. The spireme is folded into some six to eight loops.

The spireme next divides transversely into pieces. Some eight pieces at first appear, they consist of U-shaped and pot-hook-shaped lengths. The U-shaped pieces divide into pot-hooks each of which further divides transversely into two, giving in all twenty-six bodies, which come to lie upon the equatorial plane.

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EXPLANATION OF PLATE IV.

Illustrating Dr. W. Youngman's paper on Studies in the Cytology of the Hibisceae. II.

Figs. 1-33 are from the cells of root-tips.

Fig. 1. *Thespesia populnea*. Cell with its nucleus showing lengths of spireme upon the equatorial plane, division into chromosome bodies being not yet completed. $\times 2,280$.

Fig. 2. *Thespesia macrophylla*. Cell with equatorial plate of chromosome bodies. The three globules are the remains of the nucleolus. $\times 2,280$.

Fig. 3. *Eriolaena quinquelocularis*. Karyokinetic spindle at anaphase. Note the large number of minute chromosomes. $\times 2,280$.

Fig. 4. *Guazuma tomentosa*. The chromosome complement at metaphase. $\times 4,560$.

Fig. 5. *Thespesia populnea*. Longitudinal section through a spindle cutting some of the chromosome bodies at metaphase. The chromosome bodies are preparing to split longitudinally. $\times 4,560$.

Fig. 6. *Thespesia populnea*. Longitudinal section, parallel to the median plane, through a spindle at metaphase. The chromosome bodies have split longitudinally. It is to be noted that no comparison as to the size of a spindle should be made between a section as in this figure, and an entire spindle contour such as that of Fig. 3. $\times 2,280$.

Fig. 7. *Thespesia populnea*. Longitudinal section of a spindle showing chromosome bodies at anaphase. $\times 4,560$.

Fig. 8. *Thespesia populnea*. Portions of chromosome bodies at the same stage as in Fig. 5. Two of the bodies appear as if cut transversely and to show a quadripartite structure. $\times 4,560$.

Fig. 9. *Thespesia populnea*. Chromosome bodies dividing longitudinally at commencing anaphase. $\times 4,560$.

Fig. 10. *Thespesia populnea*. A cell showing a polar view of the nucleus at telophase. $\times 2,280$.

Fig. 11. *Thespesia populnea*. Cell-division. Telophase. $\times 2,280$.

Fig. 12. *Thespesia populnea*. As Fig. 10, but viewed from the vertically opposite direction, i. e. from the equatorial plane of the spindle. $\times 2,280$.

Fig. 13. *Thespesia populnea*. Cell-division. Nuclei at telophase showing as a compact mass. $\times 2,280$.

Fig. 14. *Thespesia populnea*. Cell-division. A slightly later stage than that shown in Fig. 13. The telophase mass in the nuclei is opening out. $\times 2,280$.

Fig. 15. *Thespesia populnea*. A telophase nucleus at a later stage than in Fig. 14. $\times 2,280$.

Fig. 16. *Thespesia populnea*. Section through a nucleus at telophase. The plane of the section is parallel to that of the equatorial plane of the spindle. $\times 2,280$.

Fig. 17. *Thespesia macrophylla*. As Fig. 16. $\times 2,280$.

Fig. 18. *Thespesia macrophylla*. As Fig. 16. $\times 2,280$.

Fig. 19. *Thespesia macrophylla*. A cell showing a polar view of the convex surface of a nucleus at telophase. $\times 2,280$.

Fig. 20. *Thespesia populnea*. Sections of two telophase nuclei burst by pressure on the cover-slip. $\times 2,280$.

Fig. 21. *Thespesia populnea*. Two cells showing sections of telophase nuclei $\times 2,280$.

Fig. 22. *Thespesia populnea*. As Fig. 21. $\times 2,280$.

Fig. 23. *Thespesia macrophylla*. Cell with its nucleus within which connexions between the spireme and the nucleolus are seen. $\times 2,280$.

Fig. 24. *Thespesia populnea*. As Fig. 23. $\times 2,280$.

Fig. 25. *Thespesia populnea*. Cell showing the periphery of its nucleus at telophase. $\times 2,280$.

Fig. 26. *Thespesia populnea*. As Fig. 25, but slightly later stage. $\times 2,280$.

Fig. 27. *Thespesia populnea*. Cell-division. The nuclei show the spireme loops arranged around a nucleolus. $\times 2,280$.

Fig. 28. *Thespesia populnea*. Nucleus showing chromosomes at prophase. $\times 3,420$.

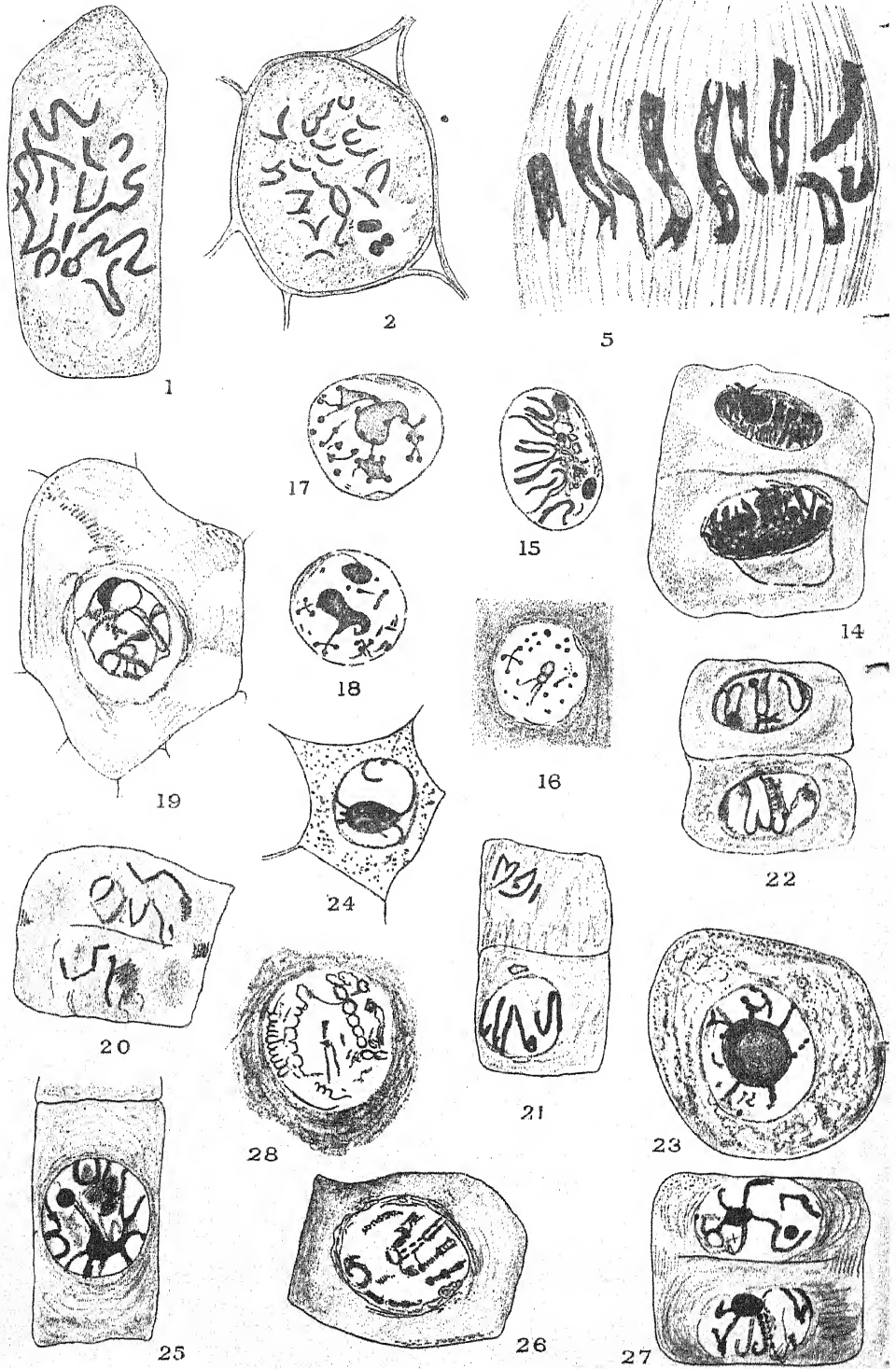
Fig. 29. *Thespesia populnea*. Cell with nucleus at prophase, stage later than Fig. 28. $\times 2,280$.

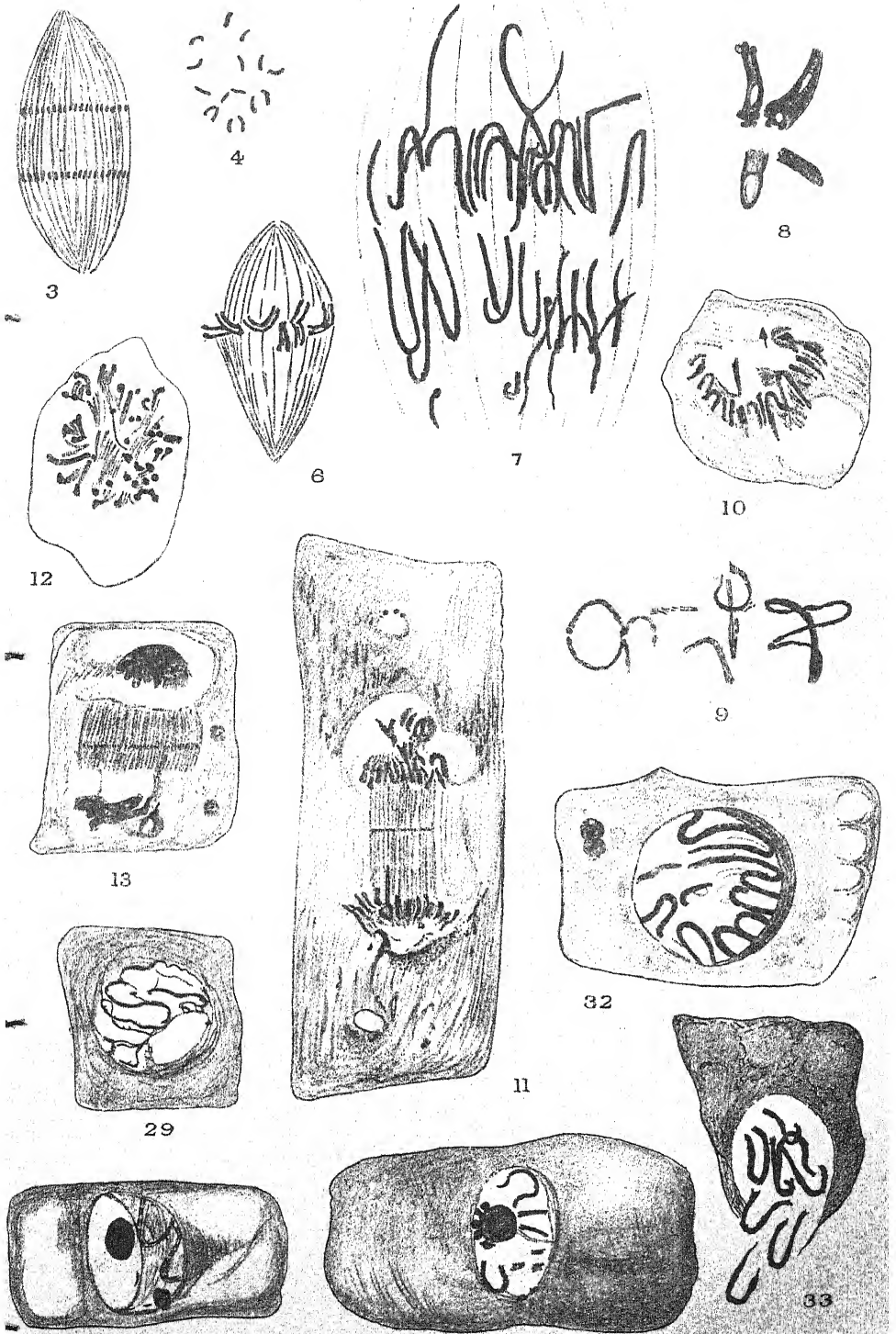
Fig. 30. *Thespesia populnea*. Cell with nucleus at prophase showing the looped spireme upon its surface. $\times 2,280$.

Fig. 31. *Thespesia populnea*. Section of cell and its nucleus. The nucleus is at prophase showing spireme loops. $\times 2,280$.

Fig. 32. *Thespesia populnea*. Section of cell, its nucleus showing the opposite pole to that of Fig. 31. $\times 2,280$.

Fig. 33. *Thespesia populnea*. Cell from the nucleus of which the loops of spireme have been swept out by the knife in sectioning. $\times 2,280$.





A Comparative Study of Growth-forms within the Species *Dermatea livida* (B. et Br.), Phillips.

BY

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With Plate V and five Figures in the Text.

INTRODUCTION.

IN recent years there has been a gradual recognition of the fact that species are not the definite units which many of the earlier workers considered them, but are in reality aggregates of an infinite number of slightly different individuals. Since most of the older specific descriptions were based upon a single specimen, it is easy to understand how so many fungi are found which do not agree exactly with any existing species. It was a difficulty of this nature which led to the present investigation, and, since the organism in question was suspected of parasitism upon various genera of the Coniferae, it was considered advisable to make a careful study of the growth-forms occurring upon the bark of these hosts. A series of such forms was finally obtained which presented all gradations between the existing species, consequently it was decided to regard them as representatives of a single, somewhat variable species, and to unite them under the oldest specific name *Dermatea livida* (B. et Br.), Phillips. The aim of the present paper has been, not only to describe the different growth-forms, but also to clear up, as far as possible, the confusion in the literature, and to provide an adequate account of the structure and development of the fungus under consideration.

HISTORICAL REVIEW.

In December 1851 Bloxam collected a small fungus on some fallen firs in Gopsal Park, Leicestershire. He sent this to Berkeley, who decided that it was a new species and named it *Patellaria livida*, B. et Br., but did not publish a description of it until 1854 (6, p. 20). In April 1852 Bloxam collected further material of the fungus from the same locality

and again sent it to Berkeley, who this time named it *P. constipata*, Berk. Some of this second collection of material was sent to Edinburgh on April 12, 1852, by Leighton (a colleague of Bloxam well known for his work on lichens) with a letter stating that it was a new fungus discovered by Bloxam and named by Berkeley *P. constipata*. This letter was read before the Botanical Society of Edinburgh, and a record of it was published shortly afterwards (1). No description of the fungus under this second name, however, appeared until Cooke's 'Handbook of British Fungi' was published in 1871 (9, p. 720), and then it was merely a brief account based on a manuscript by Bloxam which cannot now be traced. Cooke's description made no mention of the fact that Berkeley had originally named the organism, and this has led many later writers erroneously to quote Bloxam as the authority for this species. On an earlier page in the same book (9, p. 717) there is an account of *P. livida* which is almost an exact copy of Berkeley's original description. Thus these two names *P. livida* and *P. constipata* have been long regarded as representing two separate species, in spite of the fact that there is no really distinctive character mentioned in the description of either fungus. An examination of the type specimens by the present writer, however, has revealed the fact that the two organisms are identical, a conclusion also reached by Saccardo, who quoted *P. constipata* as a synonym for *Durella livida* (B. et Br.), Sacc. Although the name *P. constipata* was published before the name *P. livida*, it must be regarded as a nomen nudum since the first description of the fungus appeared under the latter name.

In 1887 Phillips (27, p. 340) published a description of the fungus in his book 'British Discomycetes'. He examined the type specimen and renamed it *Dermatea livida* (B. et Br.). During the same year Lambotte (16, p. 274) wrote a brief account of a fungus on fir wood which he named *Lecanidion lividum* (B. et Br.), Rehm, and gave as a synonym *Pezicula livida*, Sacc. Rehm, however, later disclaimed all responsibility for the former name and quoted Lambotte as the authority (28, p. 256). It was incorrect to cite Saccardo as authority for the latter combination, that name having been given by Rehm to an herbarium specimen of the fungus; it was merely quoted by Saccardo as a synonym for *Dermatella livida* (B. et Br.), Sacc.

Two descriptions of the fungus are included in Saccardo's 'Sylloge Fungorum'. The first of these (31, p. 490) occurs under the name *Dermatella livida* (B. et Br.), Sacc. and is copied from Phillips's 'British Discomycetes', reference being made both to Phillips and to Berkeley's original description. The second (31, p. 795) occurs under the name *Durella livida* (B. et Br.), Sacc. and appears to have been compiled from Berkeley's first description of *Patellaria livida* combined with Cooke's account of *P. constipata*, both of these authors being cited. The insertion of this second description must have been an oversight, but the error was

later copied by Boudier (7, p. 154), who included this species in the genus *Durella*, quoting Saccardo as authority.

In 1896 Rehm published a description of the fungus in the 'Kryptogamen Flora' (28, p. 256) under *Dermatea livida*, his account being based on a German specimen. Finally Massee (21, pp. 127, 494) examined both the type specimen and living material and renamed the fungus *Scleroderris livida*, Mass.

Meanwhile in 1870 Fuckel (11, p. 279) collected a form of this organism which he considered distinct and named *Dermatea laricicola*, Fuckel. Two years previous to this Otth (24, p. 40) had collected and described a specimen from Switzerland on 'Abies excelsa', which he named *D. Pini*, Otth, but his description was valueless for subsequent identifications owing to the immaturity of his material. Another form, named *D. Pini*, Phil. et Hark. (26, p. 22), was recorded in 1885 on pine in California.

The form which has previously been known as *Dermatea eucrita* (Karst.), Rehm was apparently first described in 1869 under the name *Peziza eucrita*, Karst., two descriptions of it being published simultaneously in the same journal, one by Karsten (13, p. 147) and the other by Nylander (23, p. 47). In the introduction to his paper Nylander mentioned a thesis of Karsten's, 'Synopsis Pezizarum et Ascobolorum Fenniae', the date of which was 1861. It is possible either that the name *P. eucrita* was used in this thesis, which unfortunately cannot now be obtained, or that it was merely a manuscript name attached by Karsten to specimens sent to the museum; this latter explanation is suggested by a foot-note in Nylander's paper. In spite of this uncertainty as to the first use of the name, it is obvious that Nylander regarded Karsten as the authority for the species. In the above-mentioned papers the descriptions of *P. eucrita* occurred under a section heading *Allophylaria*. Although no description of the fungus under the designation *A. eucrita* was ever published, the name appears to have been used on herbarium specimens, and occurred in a list of fungi published by Karsten in 1871 (14, p. 243). In the latter case Karsten gave his own name as authority, but on a specimen from his herbarium collected in 1865-66 he cited Nylander as authority. In 1871 Karsten (15, p. 166) published another description of the fungus, this time naming it *Pezicula eucrita* and stating in a foot-note that a German form *Dermatea abietina*, Awd. (4) was related to it. Saccardo (31, p. 491) examined the fungus and named it *Dermatella eucrita* (Karst.), Sacc., and finally in 1896 Rehm (28, p. 255) transferred it to the genus *Dermatea*.

For reasons to be discussed in a later section of this paper, the fungus under consideration has been regarded by the present writer as a species of *Dermatea*, and since all the forms are now to be united as one species, the oldest specific name '*livida*' has been retained, and the more recent names should be regarded as synonyms.

METHODS.

The type specimens of the existing species were, whenever possible, obtained for comparison with living material which had been collected on various hosts from a number of widely separated localities in Great Britain and on the Continent. Each living specimen, after a detailed morphological examination, was grown in pure culture. Malt agar (25 gr. malt extract, 1,000 c.c. water, 3 per cent. agar) proved the most suitable medium for this fungus, and the descriptions of the culture characters of the various forms all refer to growth upon this medium.

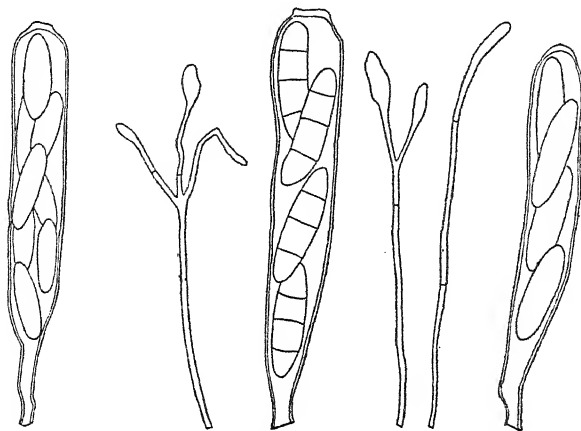
The principal points of distinction between the various forms were ascospore size and the length of the 'B' spores of the imperfect stage. In comparing the spore measurements of the different populations, statistical methods have been employed as an aid to classification.¹ The mean was taken as the average value for the character in question since it gives weight to the extreme variations, and for this reason a greater significance was attached to this figure than to the mode. In order to indicate the degree of confidence which should be placed in the results, the standard error of each mean was calculated. In comparing the means of two groups of spore measurements, the differences were taken to be significant only when they exceeded the standard error of the difference by three times, which indicates that there is a chance of 370:1 (25, Table II) against such means being identical. It may be concluded, therefore, that populations with means which diverge by this amount do not form a homogeneous group, the mean difference being too great to result solely from the operation of chance.

MORPHOLOGY.

The hyphae of this species are colourless or pale yellow, 1–3 μ in diameter, branch frequently and have comparatively numerous transverse septa. The mycelium ramifies through the cortex of the host but has not been observed to penetrate the xylem except in a few cases where the apothecia were growing directly upon an exposed surface of the wood. The hyphae become aggregated immediately below the surface of the bark to form a stroma upon which the apothecia ultimately develop. These are erumpent, and occur singly or more commonly in small groups on the bark of the tree (Pl. V, Fig. 1). They vary in diameter from 0.5–2.0 mm. are at first concave, later flat or convex, and usually have a short stalk. The hymenium is brown to orange-yellow in colour, but the peridium is distinctly paler. The asci are club-shaped, with a rather indefinite stalk,

¹ The statistical terms used are explained in 'Genetics in relation to Agriculture', by E. B. Babcock and R. E. Clausen. New York, 1927.

the apex is rounded and the wall is considerably thickened at this point (Text-fig. 1). In living material the tip of the ascus stains a reddish-brown with iodine.¹ Large numbers of asci were measured, but, since they exhibited a wide range of variability, even within a single population, it was finally decided that this character was not sufficiently constant to be

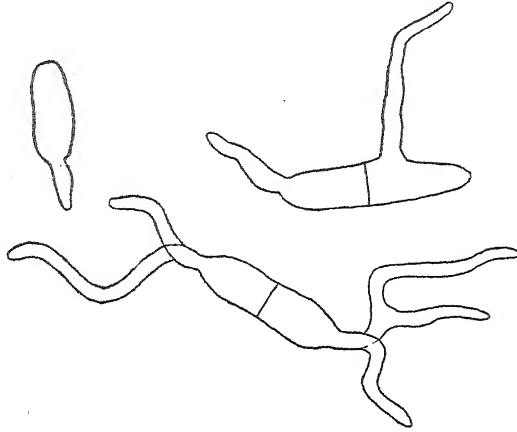


TEXT-FIG. 1. Asci and paraphyses. $\times 500$.

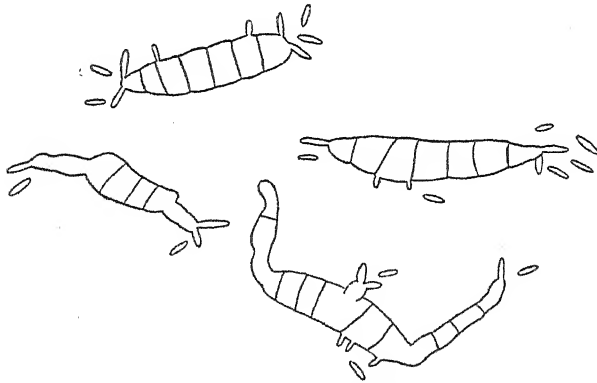
of taxonomic value. The paraphyses are about the same length as the asci, colourless, septate, often branched, and distinctly swollen at the tip; they measure approximately 2.5μ in diameter at the base and 5.0μ towards the apex. The ascospores are usually eight in number, though sometimes only four, uniseriate or irregularly biseriate, hyaline, and contain very granular protoplasm. They are at first unicellular, but may later become septate and also tend to assume a brownish tinge with age. The spores are oval to ellipsoid, straight or slightly curved, and germinate readily within 24 hours in a hanging drop of sterile water. Germination usually takes place by several germ tubes which may arise from any part of the spore, and which soon branch but do not form any transverse septa until they have attained a considerable length (Text-fig. 2). Typically, the ascospores are ejected and germinate in a unicellular condition, transverse septa are, however, frequently formed after germination, and occasionally multicellular spores may germinate. The formation, by budding, of microconidia from the ascospores was observed in certain collections of material (Text-fig. 3). A similar state of affairs has been described by

¹ Some old herbarium specimens gave a blue colouration with iodine while a few showed an intermediate purplish-brown shade. It was found possible to change the colouration from brown to violet by dehydrating some living apothecia for seventeen months at 50°C .; this demonstrates that the reaction is not constant and that it cannot, therefore, be regarded as an important diagnostic character, although reference is made to it in almost every description of *Dermatea livida*.

Bayliss Elliott (5, p. 353) and illustrated by Boudier (8, Pl. 559) in the case of *Dermatea eucrita*, and, in many respects, the observations made during the present investigation confirm the earlier account. The microconidia were produced from stunted germ tubes or directly from the ascospores,



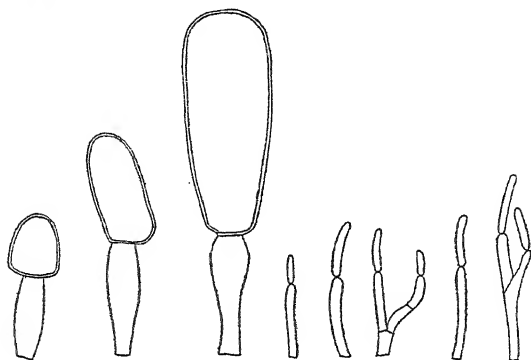
TEXT-FIG. 2. Germination of ascospores. $\times 666$.



TEXT-FIG. 3. Formation of microconidia from ascospores. $\times 666$.

but in most instances they developed from spores which had been naturally ejected from the ascus, not from spores germinating within the ascus as figured by Bayliss Elliott. The phenomenon not infrequently took place after the spores had been kept in water for several days, and was confined to spores which had formed numerous transverse septa; it has, more rarely, been observed in spore mounts from old apothecia in culture. The entire process, however, is regarded by the present writer as abnormal. The microconidia are somewhat larger and more definitely oval in outline than the smallest form of 'B' spore (which will be described subsequently in

connection with the conidial stage of this fungus). Many hundreds were retained in hanging drop cultures for more than two weeks, but in only one case was germination observed, this taking place by the formation of a single germ tube from one end of the spore. In this respect the microconidia studied by the present writer differed from those described by

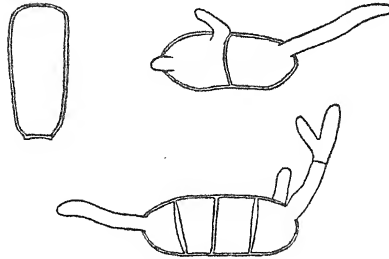


TEXT-FIG. 4. 'A' and 'B' spores with conidiophores. Immature 'A' spores are shown on the left side of the figure. $\times 1,000$.

Bayliss Elliott, who stated that the majority of such spores germinated in hanging drop cultures, either producing a small germ tube or budding off further microconidia.

Fructifications of a *Myxosporium* (Pl. V, Fig. 2) are frequently found associated with the apothecia of *Dermatea*, and culture work has shown that these two fungi are merely different stages in the life history of the same organism. Sometimes the one stage is found in abundance on a tree without any trace of the other, but usually they occur together, the apothecia often developing directly on the top of old *Myxosporium* fructifications. The latter consist of a flattened or slightly conical stroma upon which the spores are borne (Pl. V, Fig. 3); typically these spores are uncovered, but they may develop in hollows and closed cavities of the stroma. During the initial stages of its development this fructification lies beneath the surface of the bark, but later the overlying tissue ruptures, exposing the conidia. Two types of spores are produced, the one large and oblong, the other very small and filiform (Text-fig. 4). Sometimes the former are limited to the basal portion of the stroma while the latter occur over the entire surface, but often there is no such differentiation. As a rule both forms develop simultaneously, but sometimes only one is produced, or one may be formed greatly in excess of the other. When the small 'B' spores are the most abundant, it has been observed that the few accompanying 'A' spores are much reduced in size. The dimensions of the latter vary greatly, even within a single population, and therefore no importance has been attached to this character for purposes of classification.

These 'A' spores have a very distinct cell-wall and contain granular protoplasm which is at first hyaline but tends to become brown with age. They are borne on short, thick conidiophores, $12.5-16.0\mu \times 3.75-5.0\mu$, which are often slightly swollen at the tip and are seldom septate. The point of attachment to the conidiophore remains visible on the spore for some time



TEXT-FIG. 5. Germination of 'A' spores. $\times 666$.

after it has become detached, but on germination the conidium usually assumes a more ovoid outline, and this area can then no longer be distinguished (Text-fig. 5). One or more germ tubes are produced which, early in their development, branch and form transverse septa. Germinating conidia tend to become septate, the septa being usually very distinct and often thickened in the centre. Occasionally conidia may be found which have formed septa previous to germination, but this appears to be uncommon. The 'B' spores are colourless, usually straight but occasionally slightly curved, and contain clear, non-granular protoplasm. They are borne terminally on short, thin conidiophores, $10.0-17.0\mu \times 1.5-2.5\mu$, which are often branched and sometimes have transverse septa. These small conidia apparently are not capable of germination, in this respect agreeing with the 'B' spores of other genera which, with one or two exceptions, have been found to be non-functional. Several attempts were made to germinate these conidia in hanging drops of distilled water or dilute malt extract (0.5 per cent. in distilled water). After remaining for three days in the latter fluid, some of the 'B' spores produced, at one or both ends, a short process resembling a young germ tube, which, however, only attained a length of about 2μ . It is probable that this was merely a physical phenomenon arising from the absorption of water and consequent swelling of the spore contents, the swelling taking the form of a sac at the weakest place in the cell-wall.

FRUCTIFICATION DEVELOPMENT IN CULTURE.

The conidial fructification primarily consists of a small superficial, spherical stroma, usually white in colour, which enlarges rapidly and later often loses its original form, becoming irregular in outline. The young

stroma exudes drops of a transparent fluid ranging from colourless to a deep brown shade, but after some days these disappear, and soon brown, or occasionally white, waxy patches can be observed on the surface, which are due to exposure of the sporiferous layer. These gradually enlarge and often become confluent, until finally they extend over the entire surface of the stroma. The spores are usually discharged in globular masses, but sometimes long spore tendrils are formed. As a rule both 'A' and 'B' spores are developed simultaneously; in this case the spore mass is brown in colour, but, when 'A' spores only are produced, it is cream-coloured. Sections cut through stomata at various stages of their development show that the immature structures are covered by an outer layer of loosely-woven hyphae; the interior is yellow or greenish with some darker areas more or less concentrically arranged. The tissue in the centre of the dark-coloured patches gradually becomes paler (Pl. V, Fig. 4), and finally breaks down leaving a cavity surrounded by a dark brown wall, from the internal surface of which the conidiophores arise (Pl. V, Fig. 5). Thus a true multilocular pycnidium is at first developed, though later the thin outer walls break down, leaving a number of saucer-shaped structures containing spores (Pl. V, Fig. 7). The method of spore discharge depends upon the condition of the outer wall of the pycnidium at the time of spore maturity. If it is nearly complete, the spores are forced out through a very small aperture (Pl. V, Fig. 6) and form a spore tendril, but where the wall has already disappeared they form the more typical globular mass described above. Meanwhile, another series of cavities develops below the first, and these become filled with spores which are discharged as the overlying layers break down. This process is repeated until all the tissue of the primordium is exhausted.

The apothecia occur in groups upon a stroma which is, in the initial phases of its development, indistinguishable from that of the imperfect stage. The young asci are formed in closed cavities of the stroma, but later the overlying pseudoparenchyma breaks down and normal apothecia result (Pl. V, Fig. 8). These tend to be somewhat paler in colour than those occurring in nature, and the peridium is not so distinct. The ascospores produced in culture are usually slightly smaller than those formed under natural conditions, but there are indications that exposure to bright daylight during the entire period of growth of a culture may increase the mean size of the ascospores to a limited extent; exact proof of this latter point is, however, not yet available. Increased illumination also tends to accelerate the development of both the perfect and imperfect stages.

VARIATION WITHIN THE SPECIES.

(a) *Culture characters.*

During the present investigation fourteen different collections of material from various hosts were studied in culture. Numerous single ascospore isolations were made from each specimen, and, in some cases, monospore isolations also from the imperfect stage. It was found that the culture characters of each habitat collection were constant, but that variations often occurred between the individual habitat collections. On the basis of culture differences the fourteen specimens could be separated into three main groups which coincided approximately with morphological variations in spore size (see Table I).

TABLE I.

Collection Number.	Habitat.	Host.	Culture Group.	Spore measurements of collections. Mean lengths (μ) in nature.	
				Ascospore Range.	'B' spore Range.
1	Markinch	<i>Pinus</i>	I	26.5-31.0	4.7-6.3
2	Gifford	"			
3	Innerwick	"			
4	West Linton	"			
5	Newcastle	<i>Picea</i>			
6	Holland	<i>Pseudotsuga</i>	II	21.9-24.6	
7	Peebles	<i>Picea</i>			
8	Dunkeld	<i>Larix</i>			
9	Kilmun	"			
10	"	<i>Araucaria</i>			
11	"	<i>Cupressus</i>	III	20.6-21.1	7.4-8.0
12	Peebles	<i>Pseudotsuga</i>			
13	Novar	<i>Abies</i>			
14	Devon	<i>Cupressus</i>			

In Group I (Pl. V, Fig. 9) the colonies grew slowly, attaining a diameter of about 1 cm. after ten days at room temperature. The superficial mycelium was at first white, later a yellowish olive-green,¹ changing to dark drab-green at the margins. Typically, the aerial mycelium was not strongly developed, but in collection 5 it was very abundant, giving rise to a dense woolly covering of a greenish-grey colour. Fructifications occurred sporadically after cultivation for sixty to eighty-five days, the time required for the development of mature spores varying somewhat in the different populations. In collections 5 and 6 which have been in culture for over two and a half years, no pycnidia or apothecia have ever been produced.

The fungi in Group II (Pl. V, Fig. 10) grew more rapidly than

¹ The 'Répertoire de Couleurs', published in 1905 by the French Society of Chrysanthemum Growers, was consulted for the colour determinations.

those of Group I, the colonies attaining a diameter of 2 to 3 cm. after ten days at room temperature. The mycelium was of a looser texture, and the aerial stratum was well-developed. The colonies were initially white, but after about ten days their centres became a faint dark fawn colour, or, in collections 8 and 10, a faint blue-greenish grey; after thirty-eight days the colour changed to putty, with irregular patches of yellow ochre and red-brown. Within a month numerous small stromata were formed over the entire area of each colony, but fructifications were developed in comparatively small numbers, and only after the organism had been cultivated for three to four months. This group was characterized by the production of exceptionally large 'A' spores, and by the entire absence of 'B' spores. The imperfect stage was rapidly succeeded by the perfect stage.

Group III (Pl. V, Fig. 11) was practically identical in its vegetative characters with Group II, but differed markedly from it in the nature of its fructifications. Fewer stromata were produced on the surface of each colony, but they were larger and rapidly developed into pycnidia, both 'A' and 'B' spores being formed after about four weeks. The development of conidial fructifications was very profuse in this group, but the perfect stage has never been obtained, although the three collections have been in culture for over three years.

(b) *Spore size.*

The dimensions of spores are frequently used as criteria for the differentiation of closely allied species, but recent work is tending to show that, in many genera, such characters are apparently subject to considerable modification and genetic variation. This has proved to be true in the case of *Dermatea livida*, marked differences occurring in the size of spores developed both under natural conditions and in culture. Table II gives the mean lengths of twenty collections of material, which include the type-specimens of *D. eucrita* (ex Finland [specimen at Helsingfors]), *D. livida* (ex Leicestershire [specimens at British Museum (Nat. Hist.) and Royal Botanic Gardens, Kew]), *D. abietina* (ex Frankfurt a. M. [specimen at Kew]), and *D. laricicola* (on Larix, habitat not cited [specimen at Kew]). It will be seen that the mean lengths of ascospores from nature form an almost complete series, the only break occurring between the West Linton and Newcastle populations, their mean difference just exceeding three times the standard error of that difference. The series of ascospore measurements from culture was incomplete owing to the failure of some of the cultures to develop apothecia, consequently it was impossible to compare the collections one with another on this basis. A comparison of the mean ascospore lengths in culture with those of the corresponding collections from nature showed that there was frequently a significant difference between the two sets of measurements. Similar differences were found to occur between the

TABLE II.
Comparison of Spore Lengths (in μ).

Habitat.	Host.	Ascospore Measurements.					'B' Spore Measurements.				
		Nature.			Culture.		Nature.			Culture.	
		Range.	Mean with S.E.	Difference between Means.	Range.	Mean with S.E.	Range.	Mean with S.E.	Range.	Mean with S.E.	Range.
		Min.	Max.		Min.	Max.	Min.	Max.	Min.	Max.	Min.
Markinch	<i>Pinus sylvestris</i>	20.6	41.2	31.0 \pm 0.43	17.4	36.5	3.5	6.3	3.2	5.4	4.4 \pm 0.06
Gifford	"	17.8	43.4	29.8 \pm 0.41	19.3	39.6	3.5	6.0	3.5	6.7	5.2 \pm 0.07
Innerwick	"	18.4	43.4	29.3 \pm 0.33	22.2	34.6	3.2	6.0	3.2	7.0	5.2 \pm 0.08
West Linton	"	17.8	37.7	28.6 \pm 0.43	20.0	36.8	3.5	7.3	4.1	7.9	5.7 \pm 0.08
Newcastle	<i>Picea sitchensis</i>	16.2	33.9	26.7 \pm 0.38							
Holland	<i>Pseudotsuga Douglasii</i>	17.4	36.1	26.5 \pm 0.39	17.4	29.5					
Finland	<i>Pinus</i> sp. (T)	19.7	34.6	26.2 \pm 0.34							
Oxford	<i>Picea sitchensis</i>	17.4	38.4	24.8 \pm 0.34							
Peebles	"	17.4	29.5	24.6 \pm 0.31	17.4	29.5					
Norway	<i>Larix europaea</i>	19.0	30.4	24.1 \pm 0.23							
Dunkeld	" <i>europaeis</i>	17.4	30.1	23.7 \pm 0.26	17.8	31.7					
Kilmun	" <i>europaea</i>	17.1	28.2	23.2 \pm 0.21	18.1	32.7					
Leicestershire	'Fir' (T)	17.1	33.0	23.1 \pm 0.34							
Frankfurt a. M.	<i>Abies</i> sp. (T)	19.0	27.3	22.7 \pm 0.25	17.1	29.5					
Kilmun	<i>Arancaria imbricata</i> (T)	16.8	29.8	22.7 \pm 0.22							
Not cited	<i>Larix</i> sp. (T)	15.9	26.6	22.6 \pm 0.35	16.5	31.1					
Kilmun	<i>Cupressus Lawsoniana</i>	16.8	28.5	21.9 \pm 0.22							
Peebles	<i>Pseudotsuga Douglasii</i>	16.2	28.9	21.1 \pm 0.28							
Novar	<i>Abies nobilis</i>	15.1	34.9	20.9 \pm 0.34							
Devonshire	<i>Cupressus Lawsoniana</i>	15.4	31.7	20.6 \pm 0.31							

* indicates a significant difference between two collections.

(T) indicates type specimen of a previously described species.

'B' spore measurements from nature and culture, but in this case definite proof of the relationship of the two populations was necessarily lacking. Owing to the impossibility of germinating 'B' spores, the only available evidence of such relationship was the association in nature of the one group of 'B' spores with the ascospores or 'A' spores from which were derived the cultures that produced the other group of 'B' spores. For example, the Markinch collection, which is considered first in the table, consisted of apothecia and a few *Myxosporium* fructifications; the 'B' spores from the latter gave a mean of $4.8\ \mu$, while the monospore cultures grown from the associated ascospores developed 'B' spores with a mean of $4.4\ \mu$.

PARASITISM OF *DERMATEA LIVIDA*.

Various references to the parasitism of this fungus appear in the literature, but none has been supported by experimental evidence. The conidial stage was stated by Rostrup (30, p. 98: 31, p. 586) to be parasitic in Denmark on Douglas fir, Sitka spruce and larch. Neger (22, p. 126), in Germany, described the occurrence of *D. eucrita* following frost injury on spruce, and later de Hoogh (12, pp. 31 and 100) mentioned a disease of the Douglas fir in Holland attributed to the same organism. A form with small ascospores was collected in Aberdeenshire by Farquharson (10, p. 240), who considered that it was parasitic on Douglas fir, and more recently Wilson has found similar forms on living Douglas firs near Beaulieu in Invernessshire, and at Peebles. Further specimens for examination were obtained on Douglas fir from the New Forest, the Forest of Dean, and Harpenden, Hertfordshire; on *Cupressus Lawsoniana* from Devon, and Kingston Hill, Surrey; and on *Abies nobilis* from Novar, Sutherlandshire; in many of these instances, however, it appeared probable that *Dermatea* was a secondary organism following upon injury due to frost, insects or parasitic fungi.

In the hope of obtaining proof of pathogenicity, large numbers of infection experiments were carried out with the various forms of the fungus upon all the recorded hosts, but in no case was a positive result obtained. One inoculation on *Pinus sylvestris* with a culture from the Markinch collection resulted in the development of apothecia upon a small flap of bark which had been killed by the inoculation incision. Microscopic examination proved that the mycelium had not passed from the dead to the living tissue. The writer has therefore been forced to the conclusion that *D. livida* is, in general, purely saprophytic, although it may occasionally function as a weak secondary parasite.

DISCUSSION.

In considering the systematic position of the fungus described in the foregoing pages, the writer has consulted Saccardo's descriptions of *Patellaria*, Fr. and *Dermatea*, Fr. (31, pp. 786 and 550) in preference to the

original descriptions by Fries, since the latter are brief and somewhat inadequate for an exact identification. It has been stated in an earlier section of this paper that *D. livida* was originally placed by Berkeley and Broome in the genus *Patellaria*. The apothecia of *Patellaria* are defined as blackish and leathery, the ascospores as dark coloured oblong or fusoid, with two or more septa. The species under consideration does not agree with this description since the apothecia are brown and somewhat fleshy, the mature ascospores are ellipsoid and, at the time of germination, are unicellular and hyaline. These characters are, however, in complete agreement with the description of *Dermatea*, consequently the present writer confirms the conclusions of Phillips, who, as already stated, was the first to transfer this species to the genus *Dermatea*.

The question of a classification, based on spore size, of the various forms studied during this investigation should not be passed without a brief reference to the work of La Rue on *Pestalozzia guepini*, Desm. (17, 18, 19). That author has proved the existence of numerous distinct strains within the species, and has also carried out experiments on the result of selection for many generations, and on the effect of environment on spore size. He concluded that selection was totally ineffective in establishing distinct lines within pure strains, and he also found that changes of temperature and culture media did not alter spore size; the latter observation, however, does not confirm the work of Stevens and Hall (32) on other genera. La Rue (19, p. 231) writes, 'The dependability of spore measurements in delimiting species and strains is closely connected with the constancy of spore size in the forms concerned. Those who use this criterion should study carefully the variability of spore size in the forms considered'. The data cited in Table II of the present paper demonstrate that the spore dimensions of a given collection of *D. livida* are far from constant and that considerable differences may occur between the spore means in nature and in culture. Further statistical work, however, is required in order to ascertain the influence of environmental factors on the average size of the spores. Meanwhile, it is obvious that these characters alone cannot be utilized for the differentiation of species within this group of forms, thus there remains no present alternative but to unite all the forms under a single specific name. The observations on the inconstancy of spore measurements in these forms of *D. livida* bear out the recent work of various investigators who have reached the same conclusions with regard to other genera; the writer believes that further comparative studies on similar lines will only serve to emphasize the unreliability of this criterion for purposes of taxonomy. It appears probable, therefore, that in the future some new system of classification of the fungi will have to be devised, based not only on morphological but also on physiological characters; such a system has long since been found indispensable in the study

of bacteria and has proved to be practicable in that group. In the present instance it has not been considered necessary to attempt to group the numerous growth-forms of *D. livida* into physiological species, but the whole question has been discussed here in some detail on account of its possible application to other genera, which might contain dangerous parasites that were morphologically closely allied to harmless saprophytic strains.

A brief note upon the parasitism of *D. livida* was published early in 1928 by the present writer (33, p. 105). Reference was there made to a saprophytic form with large ascospores which had been identified as *D. livida*, and also to a small-spored form which appeared to be parasitic on various conifers; the advisability of regarding the latter as a new species was considered. At that time no statistical study of the growth-forms had been attempted and only a few collections of material were available. The identification of the saprophytic form was based upon the description of *D. livida* given by Rehm in Rabenhorst's 'Kryptogamen-Flora', but a subsequent examination of the type specimen revealed the fact that this description, which had been prepared from a German collection, was inaccurate with regard to spore dimensions, and that the supposed parasitic form approached more closely to the type specimen than did the saprophytic strain. This matter, however, has ceased to be of importance since all the forms have now been regarded as one species.

The imperfect stage of *D. livida* has been identified as *Myxosporium abietinum*, Rostr. (20, p. 480:29, p. 98:30, p. 586), but it differs in one respect from the description of that fungus, namely, in the presence of 'B' spores. An attempt was made to obtain Rostrup's type specimen for comparison, but it could not be traced. In such a case it is obviously impossible to state conclusively that the two organisms are identical, but there is at least a high degree of probability that this is so, for it would be comparatively easy to overlook the 'B' spores if these were only present in limited numbers. The existence of an imperfect stage with two types of conidia has long been recognized in certain species of *Dermatea*, but this appears to be the first time that it has been definitely identified with *Myxosporium*, although Lind (20, p. 133) suggested a resemblance to that genus in the case of a conidial form associated with *D. quercina* (Fuckel), Rehm. The same writer further mentioned that *Stilbella Rehmiana*, Rabh. was considered to be the imperfect stage of *D. eucrita* (now regarded as synonymous with *D. livida*), but that Rostrup had, according to his diaries, believed the conidial stage of that species to be *Micropera abietis*, Rostr. Neither *Stilbella* nor *Micropera*, however, show any resemblance to the true imperfect stage of *D. livida* which, in the course of the present investigation, has been conclusively proved to be of the *Myxosporium* type.

The fructification of the imperfect stage is typically a simple acervulus,

but it may exhibit a more complicated structure due to the formation of deep hollows and even closed spore-bearing cavities in the stroma. The observations on the development of these fructifications in culture show that, at least under artificial conditions, a true pycnidium is initially produced. A similar state of affairs has been described by Archer (3, pp. 66 and 67:2) in the case of two species of *Pestalozzia* and in *Asterosporium Hoffmanni*. These facts demonstrate the impossibility of drawing a firm line of demarcation between the two types of fructification, a point of considerable taxonomic importance, since it is on this character that two of the main groups of the Fungi Imperfecti are separated.

SUMMARY.

1. The growth-forms of *Dermatea* occurring upon the bark of conifers have been compared; this comparison was based upon cultural characters and a biometric study of spore size. The existing species, viz. *D. livida*, *D. eucrita*, *D. laricicola*, *D. abietina*, *D. Pini*, occupy various positions in one continuous series of forms, consequently these have been united under the oldest specific name, *D. livida*, and the more recent names should now be regarded as synonyms.

2. The conidial stage of *D. livida* has been identified as *Myxosporium abietinum*. In addition to the large conidia described by Rostrup, minute, non-germinating 'B' spores were found. The length of these 'B' spores varied slightly in the different forms.

3. An investigation of the development of the conidial stage in culture showed that a true pycnidium is initially produced, the upper wall of which ultimately breaks down, leaving a saucer-shaped structure containing spores. Indications of a similar condition in nature were observed.

4. All infection experiments with these fungi have given negative results, but field observations suggest that certain of the forms with small ascospores may occasionally function as weak secondary parasites.

In conclusion, the writer wishes to express her thanks to Dr. Malcolm Wilson of Edinburgh University, and to Miss E. M. Wakefield of the Royal Botanic Gardens, Kew, for their helpful advice during the course of this work. The Director of the Royal Botanic Gardens, Kew, the Keeper of the Botany Department, British Museum (Nat. Hist.), and Professor Elfving of Helsingfors kindly gave facilities for the examination of the necessary type specimens. Thanks are also due to Mr. J. de Hoogh and Mr. H. van Vloten of Wageningen, who assisted the writer to make field observations on this subject in Holland; also to Dr. G. G. Hahn (New Haven, Conn.), Mr. J. Macdonald (Edinburgh), and Mr. J. S. L. Waldie (Reading), who kindly supplied material and field notes.

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EXPLANATION OF PLATE V.

Illustrating Dr. Mary J. F. Gregor's paper on a Comparative Study of Growth-forms within the Species *Dermatea livida* (B. et Br.), Phillips.

Fig. 1. Apothecia of *Dermatea livida* on bark of Douglas fir. $\times 2$.

Fig. 2. Fructifications of *Myxosporium abietinum* on branch of Douglas fir. $\times 1$.

Fig. 3. Transverse section through a fructification of *M. abietinum*, showing conidia formed in hollows and closed cavities of the stroma. $\times 45$.

Figs 4-7. Stages in development of conidial fructification in culture.

Fig. 4. Immature pycnidium, interior composed of light-coloured, thin-walled pseudo-parenchyma. $\times 150$.

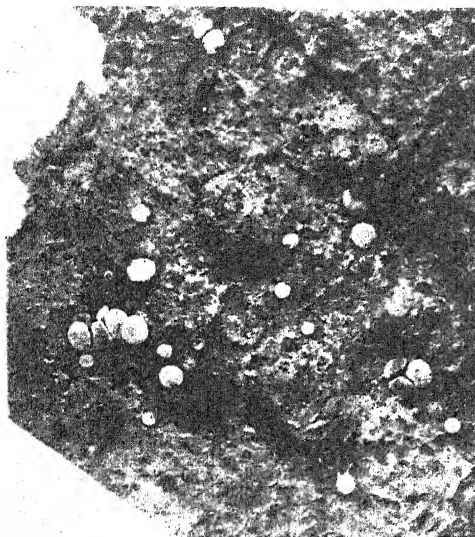
Fig. 5. Pycnidium containing mature spores. $\times 150$.

Fig. 6. Older pycnidium with outer wall breaking down. $\times 134$.

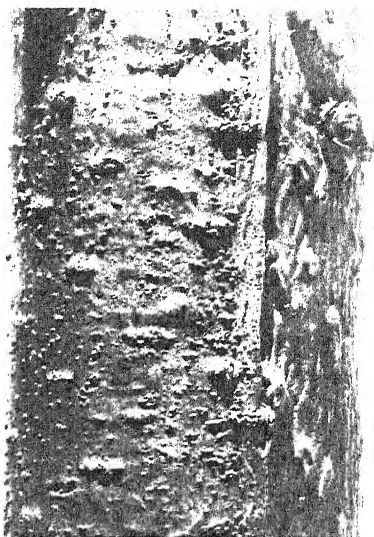
Fig. 7. Exposed sporiferous layer after disintegration of outer walls of pycnidium. $\times 100$.

Fig. 8. Development of perfect stage in culture: a group of young apothecia is seen on the left and a mature apothecium on the right. $\times 100$.

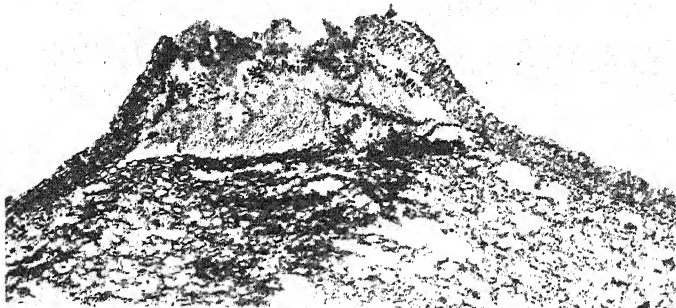
Figs. 9-11. Representative cultures of the culture groups I, II, and III. Description in text.



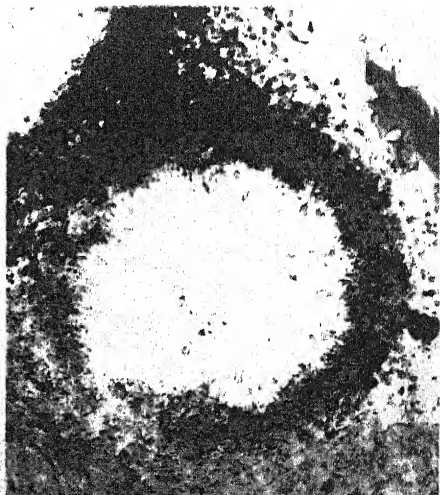
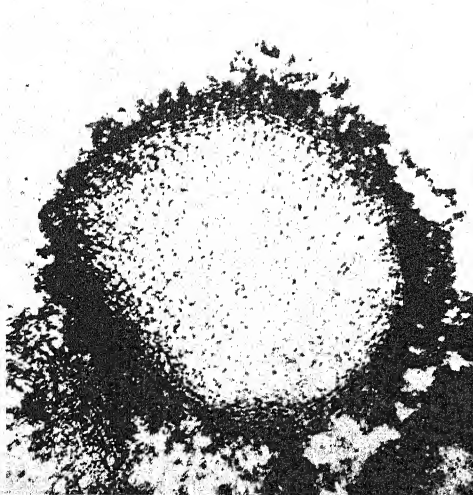
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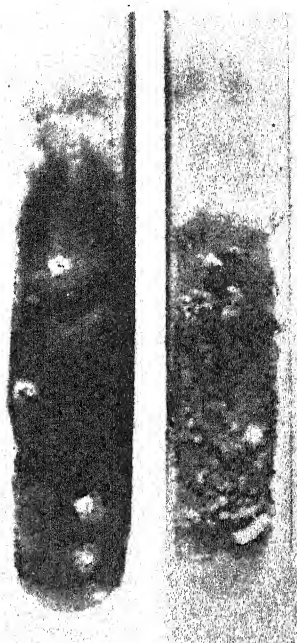
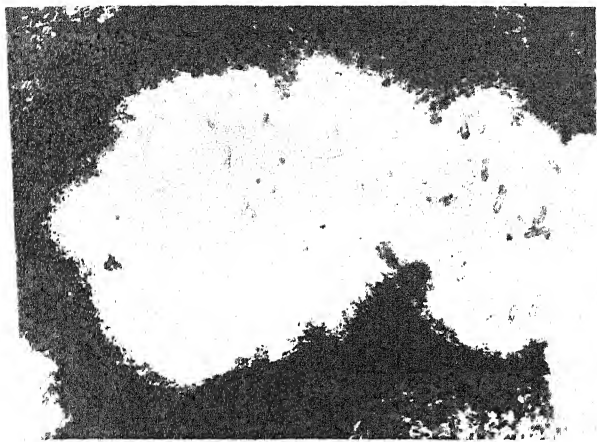


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On Carpel Polymorphism. IV.

BY

EDITH R. SAUNDERS.

(*Sometime Fellow of Newnham College, Cambridge.*)

With forty-five Figures and four Diagrams in the Text.

THE present account deals with the Sterculiaceae. This family was selected for investigation since it appeared, according to current description, to show not only certain anomalies in the gynoecium, but also various inconsistencies in the disposition of the several floral whorls. It includes many genera, with flowers isomerous throughout. The full androecium is 2-whorled, and on the present view of carpel polymorphism the gynoecium also consists of 2 whorls. In the pentamerous forms we shall therefore write the floral formula in the simplest cases (Buttnerinae) $K_5 C_5 A_5 + 5 G_5 + 5$. Such genera, as is often the case in flowers with this type of ground plan, consistently exhibit the anomaly of obdiplostemony associated with an antepetalous disposition of the loculi (compare e. g. the Geraniaceae, Oxalidaceae, and Ericaceae with its allies). A further complexity frequently met with in the Sterculiaceae, but not present in the other families cited above, is that of deduplication in the androecium. The stamen complement on the radii of the petals is in such cases doubled, trebled, or even quintupled; the antesepalous members, which are reduced to staminodes, may also show the same feature in a lesser degree (*Commer-sonia* and (occasionally) *Fremontia*, see Fig. 45). These variations in the androecium scheme do not, however, affect the positional relations of the gynoecial whorls, since the whole vascular mass supplying each antepetalous stamen group leaves the central cylinder as a single bundle complex, often conjoined with the complex for the corresponding petal, only breaking up into the component strands after its exit. The two genera *Hermannia* and *Dombeya*, both possessing calyx and corolla are, however, commonly cited as exceptions to the general sterculiaceous scheme in having the loculi in line with the sepals. This is unquestionably the case in *Hermannia*, where the complete suppression of the antesepalous stamen whorl has the effect of bringing about a shift of the sterile carpels and loculi from the usual antepetalous to an antesepalous position. This compensatory adjustment

does not, however, remove the characteristic condition obtaining in typical obdiplostemony, for the one staminal whorl which is present stands here, like the outermost of the two whorls where both develop, in line with the petals. That is to say, the law of alternating whorls is transgressed equally in *Hermannia* with its one staminal whorl as in those more typical genera with a two-whorled androecium. *Dombeya*, in which both androecium whorls reach development, appears at a first glance to exhibit a near approach to the diplostemenous condition. This divergence from other similarly constituted sterculiaceae genera is, however, apparent rather than real. The vascular elements for a petal and a fertile stamen-group leave the central cylinder on the *same* radius, more or less as one mass. That is to say, petal and fertile stamens are at first superposed. But there is not sufficient space on the original radius for the development of a large petal and, it may be, a quintuplet of stamens. The petal becomes asymmetrical, the stronger growth of one half causing the point of exertion to become shifted to one side. This lateral trend involves to some extent some members of the superposed stamen group as they become spread out in the tangential plane. This lateral displacement accounts for the discrepancy between Eichler's description and diagram (2), in which the original relations are correctly represented, and the statements of Baillon (1) and K. Schumann (7, 8), who interpret the intermediate position assumed later by petals and fertile stamen groups as a case of diplostemony. Any doubt on this point is removed by the course of the further development. The vascular cords for the sterile carpels, which stand in line with the loculi, originate on the same radius as the whole complex for each petal and fertile stamen group, those for the antesealous staminodes on the alternate radii. This asymmetrical development of the petals in *Dombeya* as compared with *Hermannia* goes hand in hand with an asymmetrical venation scheme. Whereas in *Hermannia* each petal receives a mid-vein and twin laterals and develops symmetrically, appearing horse-shoe-shaped in a cross-section of the base, in *Dombeya*, the whole venation system sweeps round to one side in conformity with the lateral torsion, and the consequent displacement of the exertion point from the angles of the pentagonal andro-gynoecium structure occupied by the stamen-groups to a position nearly half way along the flat sides.

Thus it appears that whether two staminal whorls are developed, as is usually the case, or whether only one, as in the exceptional genus *Hermannia*, the law of alternation of whorls is always violated in the succession from corolla to androecium. This condition is due to the same cause here as in the case of the Geraniales already investigated (5, 3), viz. to dimorphism of the members of a 2-whorled gynoecium, the antesealous (in *Hermannia* antepetalous) carpels remaining more central, while the antepetalous (in *Hermannia* antesealous) members curve outwards to permit

of the formation of the loculi. This dimorphism, as will appear from the following description of various individual genera, explains certain other structural features which would otherwise present some difficulty.

BUTTNERINAE—THEOBROMINAE (Figs. 1–5 and Diagram A)

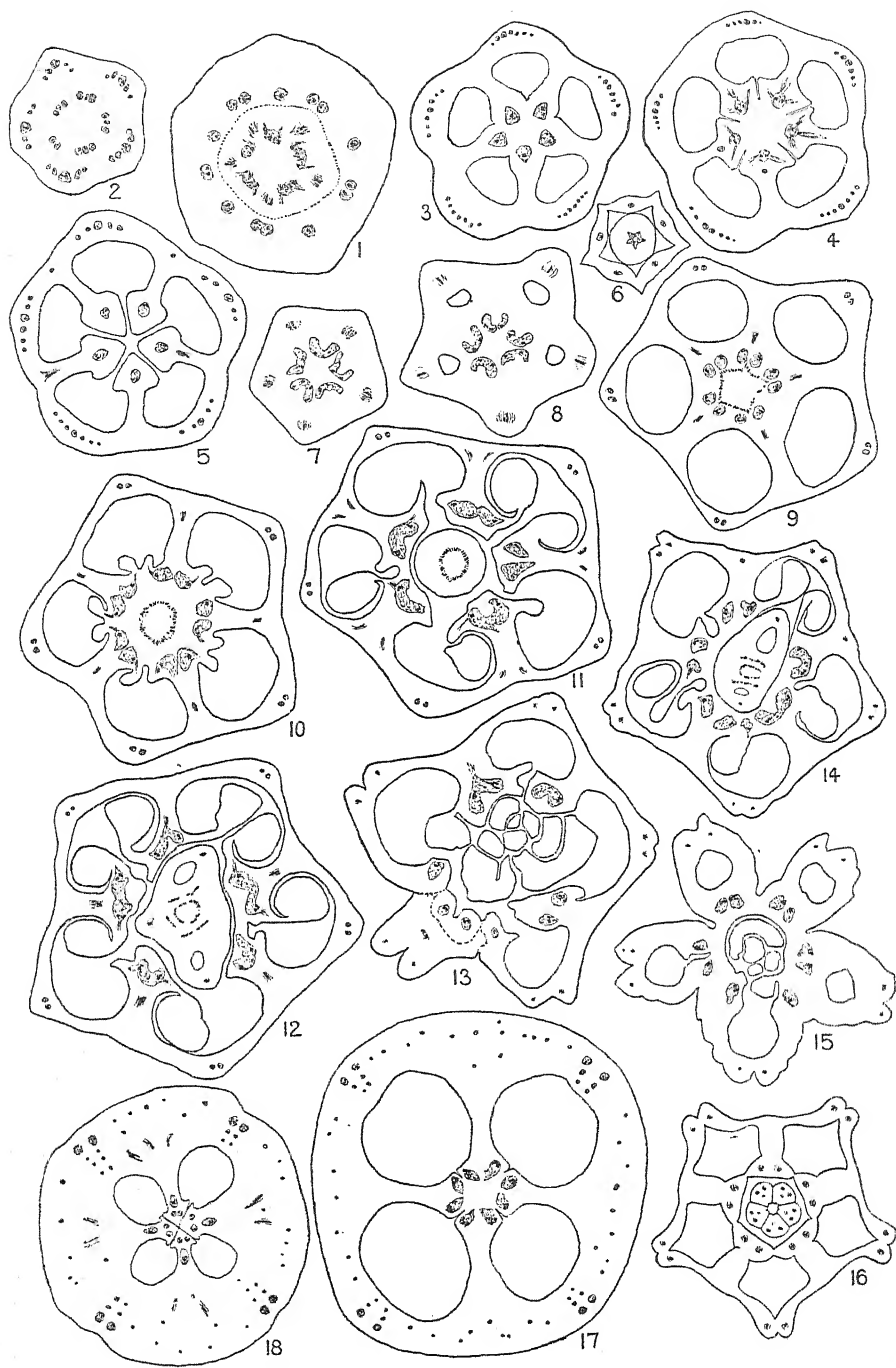
Flowers ♂, obdiplostemenous, calyx and corolla present, functional stamens single, loculi antepetalous.

Theobroma Cacao, L. K₅ C₅ A₅ + 5G₅ + 5. Transverse sections taken through the flower base just above the level at which the perianth tube becomes free show a central vascular cylinder from which 5 single and 5 double bundles emerge on alternate radii, both sets coming to be arranged in a single ring (Fig. 1). The single bundles run to the 5 episepalous staminodes, the double bundles to the antepetalous stamens. The residual vascular elements become reformed into a ring from which there presently emerge on the radii of the stamens 5 bundles, which become the midribs of sterile carpels, leaving in the centre 5 alternate vascular masses, which serve the fertile members (Fig. 2). Lateral strands from these masses arise at once, and turning outwards into the ovary wall, become grouped on either side of the sterile carpel cords in such a way as to give these carpels a false appearance of being of typical valve form (Figs. 2, 3). Later, the five fertile carpel cords left in the centre break up, giving rise to a median bundle which runs out a short distance in the 'septum' before turning upwards, and two lateral portions which remain central, and fuse to form a single bundle. This bundle supplies the near row of ovules in the loculus on either side. About this level lines of cleavage develop, extending towards the centre from the inner angle of each loculus (Fig. 4) demarcating the ventral surface of the fertile carpels, which at lower levels merge, undefined, with each other and with the pith. As the pith disappears the cleavage becomes complete, and the fertile carpels become free from one another along their whole inner face (Fig. 5). The style-column is terminal, ending in five stigmatic arms centred over the antepetalous carpels. Differentiated vascular elements cease below this level, but the position of the arms and the antecedent division of the fertile antepetalous carpel-cord at the style-base into twin bundles indicates that each arm is the prolongation of $\frac{1}{2}1\frac{1}{2}$ carpels.

HERMANNIEAE (Figs. 6–16 and Diagram B).

Flowers ♂, calyx and corolla present, stamens single, antepetalous (= the position in typical obdiplostemony), staminodes absent, (hence) loculi antepetalous.

Hermannia althaeifolia L. (*H. plicata*, Ait.), *H. cuneifolia*, Jacq., *H.*



FIGS. 1-18. All from transverse sections taken, when in series, at successively higher levels.

1-5. *Theobroma Cacao*, L. 1. Flower base above the level of exsertion of the perianth. Towards the periphery the single and twin bundles for the antesepalous and antepetalous staminal members respectively. The area of the ovary is defined but not yet disjoined from the androecium ring. The five vascular cords for the antepetalous carpels are turning out from the centre; the bundle masses for the antesepalous members, from which strands are already arising to pass into the ovary wall, are seen nearer the centre on the alternate radii. 2. Gynoecium base below the level of the loculi. The outgoing strands seen in 1 are ranged beside the antepetalous carpel cords; the more central strands of each antesepalous carpel are not yet condensed into a single main cord. 3. The gynoecium after the appearance of the loculi, and the formation of the antesepalous carpel cords. 4. The same in the ovule-bearing region (ovules not shown). The antesepalous carpel cords show differentiation into a median strand which has turned out towards the periphery and an inner strand from which branches pass to the ovules. 5. The same above the ovule-bearing level. The pith has come to an end and the antesepalous carpels are now completely disjoined at their inner face. The outer median bundle of two of these carpels (below on the left and right) has begun to turn towards, and in the front member has already become again merged with the corresponding fertile strand.

6-15. *Hermannia plicata*, Ait. (from a specimen showing proliferation). 6. The androecium tube consisting of five antepetalous stamens surrounding the gynoecium with a central vascular ring and pith. 7. Gynoecium base below the level of the loculi, showing the five antepetalous carpel cords towards the periphery, and those of the antesepalous members on the alternate radii in the centre. 8. The same at the level of origin of the loculi. 9. The gynoecium just below the ovule-bearing region. The cords of both the antepetalous and the antesepalous carpels have given rise to twin bundles. In the latter case a median strand has also been differentiated and is turning outwards. In the centre numerous superfluous vascular elements are being reformed into a ring. 10. The same from the ovule-bearing region (ovules not shown). 11-13. The same showing stages in the development of spirally arranged, supernumerary carpels on the proliferating axis, with consequent disturbance of the normal symmetrical arrangement. 11. In the centre the proliferating axis, now free, below the level of origin of the supernumerary carpels. 12. The midribs of three of these additional carpels have turned out successively from the newly-formed ring to permit of the formation of the loculi, of which two have already made their appearance. 13. After formation of several of these additional carpels, now exserted and open owing to the margins failing to unite after becoming free from the pith. (For the sake of simplicity the ovules which are borne on the margins are omitted.) 14, 15. Showing a similar case of proliferation from another flower. 16. *H. pallens*, Eckl. and Zeyh. The gynoecium just before the loculi become closed, showing the slightly 'gynobasic' style column free in the centre (from a normal flower). The connate style filaments which stand in line with the loculi each show a central core of conducting cells and twin vascular strands = the twin bundles of the sterile carpel midrib which in its inward course over the top of the closed loculus has picked up the half cord of the neighbouring fertile carpel to right and left respectively. 17, 18. *Fremontia californica*, Torr. 17. The gynoecium in the ovule-bearing region (ovules omitted) showing the twin bundles and twin series of bundles of the fertile and sterile carpels respectively. 18. The same above the ovule region. The fertile carpels are now delimited at their inner face, the pith having come to an end (from a tetramerous specimen). [Figs. 1 and 2 are orientated in the usual manner (axis posterior); in Figs. 3-18 the orientation is reversed (axis anterior)].

depressa, N.E.Br., *H. linifolia*, Burm., *H. pallens*, Eckl. and Zeyh., *H. vesicaria*, Cav. K5 C5 A0 + 5 G5 + 5. The whorl of fertile stamens is superposed on the petals, as in other members of the family; the antesepalous whorl is here completely suppressed (Fig. 6), and the loculi in consequence make their appearance on the radii of the sepals.

In transverse sections through the flower base after the exit of the vascular trunk cords supplying the perianth and antepetalous stamens, the outline of the gynoecium appears defined as a pentagonal structure with the points in line with the sepals and the flat sides centred on the petal-stamen radii. Five bundles from the residual central cylinder turn outwards in line with the angles of the pentagon, and become the main cords of five sterile carpels. The remainder of the vascular tissue becomes condensed into five bundle masses on the alternate radii, and these become the cords of five fertile carpels (Fig. 7). The loculi make their appearance in line with the sterile cords (Fig. 8), and the further development follows the same general course as in *Theobroma* (described above). The slightly 'gynobasic' style-column shows at its fluted base the five component filaments (Fig. 16). These stand over the loculi and remain conjoined to the top, where they terminate in a single common stigma. The twin bundles of each sterile carpel midrib curve inwards over the top of the loculus as it closes, each bundle picking up the half cord of the fertile carpel on the corresponding side in its course. The resulting conjoined strands come to an end at the base of the style-column, the column itself showing no differentiated vascular tissue.

In the case of *H. plicata* it chanced that all the flowers which were examined were from the same individual, and all showed the same peculiarity—proliferation of the axis with formation of additional carpel members and consequent considerable disturbance of the usual two carpel whorls (Figs. 9–15). In none of the flowers was the whole of the residual vascular tissue utilized, as is ordinarily the case, in the formation of the normal gynoecium, the unused portions becoming reformed afresh into a central ring which served to supply several additional carpels. These supernumerary members are arranged spirally. In the case of each such member a bundle turns out from the reformed cylinder to become the midrib, and a loculus makes its appearance. As development proceeds the carpel edges become disjoined from the axis, but do not unite. The carpel, therefore, has the form of an open valve, and bears ovule-like structures on its free margins. It is noteworthy that with the reversion from a whorled arrangement and a syncarpous condition to a spiral arrangement and apocarpy, the carpels cease to show polymorphism and uniformly revert to the primitive valve type. We meet with somewhat similar change from the polymorphic to the monomorphic condition in the single and double flowers of *Kerria japonica* (4), p. 604, Figs. 197–9.

DOMBEYEAE (Figs. 19-30 and Diagram C).

Flowers ♂, obdiplostemenous, calyx and corolla present, functional stamens in groups, loculi antepetalous.

Dombeya Burgessiae, Gerr., *D. Dregeana*, Sond., *D. spectabilis*, Boj., *D. umbraculifera*, K. Schum., *D. Wallichii*, Benth. and Hook., *D. Cayeuxi* (garden form), $K_5 C_5 A_5 + 5_{2-6} G_5 + 5$. As stated above (p. 92), the peculiar asymmetry of the venation system of the corolla, resulting possibly from the deduplication of the antepetalous stamen members causes the point of exertion of the individual petals to be displaced from its true position in line with the loculi, an asymmetry which is partially rectified as the free limb becomes fully developed. With both staminal whorls present typical obdiplostemony obtains in the early stage of development, the cords for the antesepalous staminodes leaving the central cylinder later than the vascular complex serving each petal and superposed stamen group. The gynoecium is constructed on the same ground-plan as that of *Hermannia*. At the ovary apex the twin bundles of the sterile carpel cord turn inwards over the top of the loculus, and merge with one half cord of the fertile carpel to right and left respectively. The resulting pair of strands usually enter the base of the slightly 'gynobasic' style column fuse into one bundle, and continue for a longer or shorter distance up the column, which ultimately branches into five stigmatic arms standing over the loculi and in line with the petals.

STERCULIEAE (Figs. 31, 32, 44 and Diagram D).

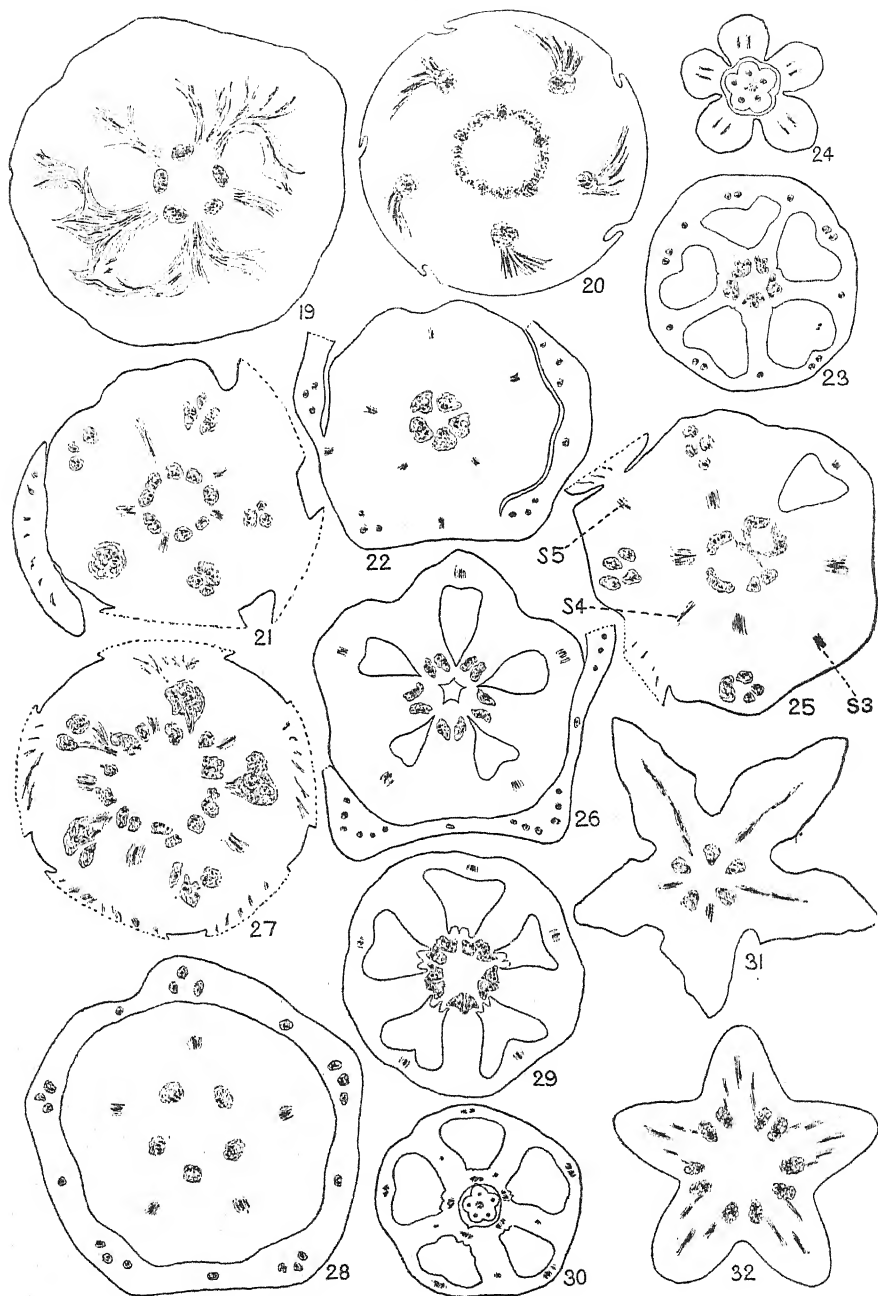
Flowers structurally ♂ but functionally unisexual, androgynophore present, perianth members in the form of tepals,¹ stamens in two whorls, those in line with the tepals in bundles, those on alternative radii single, loculi in line with the tepals.

Sterculia (Firmiana) platanifolia, L., *S. diversifolia*, G. Don., *S. (Pterygota) alata*, Roxb., *Brachychiton populneum*, R. Br., *Cola acuminata*, R. Br.

The genera included in this section are functionally unisexual, but the non-functional sex organ is always developed to some extent. They are further distinguished from those of the preceding sections in that the partially gamophyllous perianth is single, with valvate aestivation. It is usually coloured, and has been regarded as a petaloid calyx, the corolla being apparently absent. A distinct androgynophore is present, and is often of considerable length, so that the levels of emergence of the vascular cords for the perianth and the androecium are far apart.

A study of the vascular anatomy throws a new light on the nature of

¹ The term is here used in its aptest sense to indicate perianth structures constructed from tissue material which is utilized in forms with a two-whorled perianth for the formation of a distinct calyx and corolla (see later, p. 100).



FIGS. 19-32. All from transverse sections taken, when in series, at successively higher levels.

19-24. *Dombeya Burgessiae*, Gerr. 19. Flower base at the level of emergence of the five vascular bundle masses which break up to furnish the cords for the sepals, petals, and antepetalous stamen groups. 20. The same after exsertion of the sepals. Towards the periphery the five fan-like branch systems for the five petals, becoming differentiated from the five bundle masses serving the antepetalous stamen groups (the petals at first show very marked unilateral development according with the contorted aestivation). In the centre the residual vascular ring for the antesealous stamens and the gynoeceium. 21. The same after exsertion of the petals (one free petal is seen in position on the left). The five bundles for the antesealous staminodes are seen turning out from the residual central ring. Towards the periphery the bundle masses serving the stamen groups. 22. The gynoeceium partly surrounded by the androeceium ring, from which it is now disjoined except on the lower side (the median upper sector of the ring has been cut away). The single bundles for the antesealous staminodes are seen alternating with the bundle groups for each of the antepetalous stamen triplets. The vascular cords for the five antepetalous sterile carpels have moved out towards the periphery, leaving in the centre the five bundle masses for the fertile antesealous members. 23. The gynoeceium after complete separation from the staminal tube. The loculi have made their appearance in line with the sterile carpel cords, which have divided into twin bundles. On the alternate radii the vascular system of the fertile carpels consists of a bundle which has turned outwards and now runs upwards in the ovary wall, and in the centre a bundle mass which gives rise to the branches supplying the ovules. 24. Apex of the ovary with the slightly 'gynobasic' style column in the centre. The twin bundles forming the main cord of each antepetalous carpel are seen turning inwards over the closed loculus. As they turn downwards to enter the base of the style column they pick up the half cord of the fertile carpel to right and left respectively and condense into the single strand seen in each component filament of the column. In the centre a single core of conducting cells. 25, 26. *D. Wallichii*, B. and H. 25. Flower base after exsertion of the sepals, cut slightly obliquely. On the right two stamens, two staminodes and three petals have already been exserted and are not seen. The exsertion radius of the two remaining petals (cut away) has become shifted through torsion from the radius of origin (radius of a stamen group) to the radius of a staminode. The trunk cords for the three remaining stamen groups have begun to break up into the separate bundles for the individual stamens. The single bundles for the three staminodes not yet exserted (S_3, S_4, S_5) are seen cut longitudinally on the alternate radii. The midrib bundles for the five sterile carpels which have turned out from the central ring are seen similarly cut; those for the fertile carpels are in process of becoming differentiated. Only one loculus has as yet made its appearance. 26. The ovary and a portion of the staminal tube. In the staminal tube the single bundles for the staminodes alternate with six symmetrically arranged bundles for each stamen group. All the loculi have now made their appearance in line with the antepetalous carpel cords. In the centre the antesealous carpel cords appear as twin bundles. The pith at this level has come to an end, leaving the inner face of the fertile members exposed. 27-30. *D. Dregeana*, Sond. Flower base above the level of exsertion of the calyx. At the periphery the fan-like vascular systems of the five petals which have been cut away. The exsertion midpoint is becoming shifted from the radius of the stamens to that of the staminodes. The five single bundles for the antesealous staminodes have left, or are leaving, the central ring. Further out on each alternate radius is the vascular complex for one of the stamen groups. 28. The ovary base surrounded by the staminal tube showing the five sterile carpel cords passing to the periphery, and in the centre on the alternate radii the five cords for the fertile carpels. 29. The ovary after the appearance of the loculi. The fertile carpel cords show a stage intermediate between that seen in Figs. 26 and 23. 30. The same above the ovule level. In the ovary wall the sterile carpel cords of twin strands; on the central side of each loculus twin bundles = the half cords of the fertile carpel to right and left, which are picked up by the twin sterile strands as they turn in over the closed loculi to enter the style column. In the centre the slightly 'gynobasic' style column as in Fig. 24. 31. *Cola acuminata*, Schott. and Endl. Ovary base showing the bundles which become the midribs of the sterile carpels turning out from the central cylinder; on the alternate radii the cords for the five fertile carpels. 32. *Sterculia (Firmiana) plataniifolia*, L. Gynoeceium base. In the middle line of each protuberance the sterile carpel midrib is seen cut longitudinally as it turns out to the periphery. In the centre on the alternate radii the twin vascular masses of the five fertile carpels. Lateral branches from these masses have already begun to pass outwards; these branches furnish the whole venation system of the ovary wall except for the unbranched sterile midribs. [Figs. 20 and 32 are orientated in the usual manner (axis posterior), in the remaining figures the orientation is reversed (axis anterior)].

this single perianth. In the simplest case, as e. g. in *Cola acuminata*, R.Br., five vascular cords turn out from the central cylinder at the flower base on one set of radii, and are immediately followed by another set of five on the alternate radii. These ten cords are homologous with the ten equidistant cords seen, e. g. in *Theobroma*, which in a pentamerous type having both calyx and corolla, furnish the midribs of the sepals and petals. In *Cola* the earlier-formed five cords follow the normal course, becoming the midribs of the five existing perianth segments. The other five cords continue upwards on the alternate radii nearly to the level at which these members separate. They then bifurcate, the one half cord passing into the free perianth segment on one side, and continuing upwards as a lateral vein, the other half cord passing similarly into the free segment on the other side. *Sterculia* and *Brachychiton* show the same arrangement, and although in these two genera anastomoses between the cords immediately after their exit from the central cylinder somewhat obscures the ground plan at this level, it becomes clear as soon as the cords reach the periphery, and turn upwards. It is thus plain that the perianth members in the Sterculiaceae are not the counterparts of single individual sepals. The calyx (so-called) is evidently supplied with vascular tissue, which should suffice—and no doubt did in some ancestral type—for both calyx and corolla. But the original plan has undergone modification. Instead of a two-whorled scheme each member of the single whorl receives the vascular cord proper to itself—the cord lying on its own radius—and, in addition, half of the adjacent cords to right and left on the alternate set of radii—half the cords, that is to say, which should properly serve neighbouring petals. We are in fact dealing here with what might be described, if we may be allowed the expression, as a synthetic product. This double origin of the perianth accounts for its general petaloid character, and for the particular colour distribution seen in such a form as *Sterculia discolor*, F. Muell., in which anthocyanin occurs in broad marginal strips, and also over part of the inner face, but is absent from the middle portion of the back, the whole presenting the appearance of a red petal with a smaller brown sepal plastered on the back.¹ This semblance is further heightened by a similar localized distribution of characteristic hairs which occur on both surfaces, but are restricted to the central area, and are lacking on the coloured borders. These perianth members may with special aptness be described as tepals. An instance of the same type of synthetic construction is met with in a member of the Leguminosae (6), p. 236. In *Saraca indica* the perianth is utterly unlike that

¹ The free edges of the perianth segments are strongly induplicate in the bud in this species. It might therefore be questioned whether this circumstance in itself did not account for the scheme of colouration since a different physiological condition might well obtain in the intumed, applied surfaces as compared with the exposed region. That the colour distribution cannot be due to this cause alone is shown by the fact that in other species which are strictly vulvate in the bud the tepals are coloured on the inner face.

of a typical papilionaceous flower. Instead of the usual pentamerous calyx and papilionaceous form of corolla it shows two pairs of thick, coloured structures, the one pair being slightly larger than the other. The members of the larger, median pair are each, in reality, triple structures, the anterior one corresponding to a median sepal and the two neighbouring petals, the posterior one to the median petal and the two neighbouring sepals. Each member of the smaller, lateral pair similarly corresponds to one sepal and one petal. Thus there arises a single cruciform perianth whorl where we should ordinarily expect a distinct calyx and papilionaceous corolla.

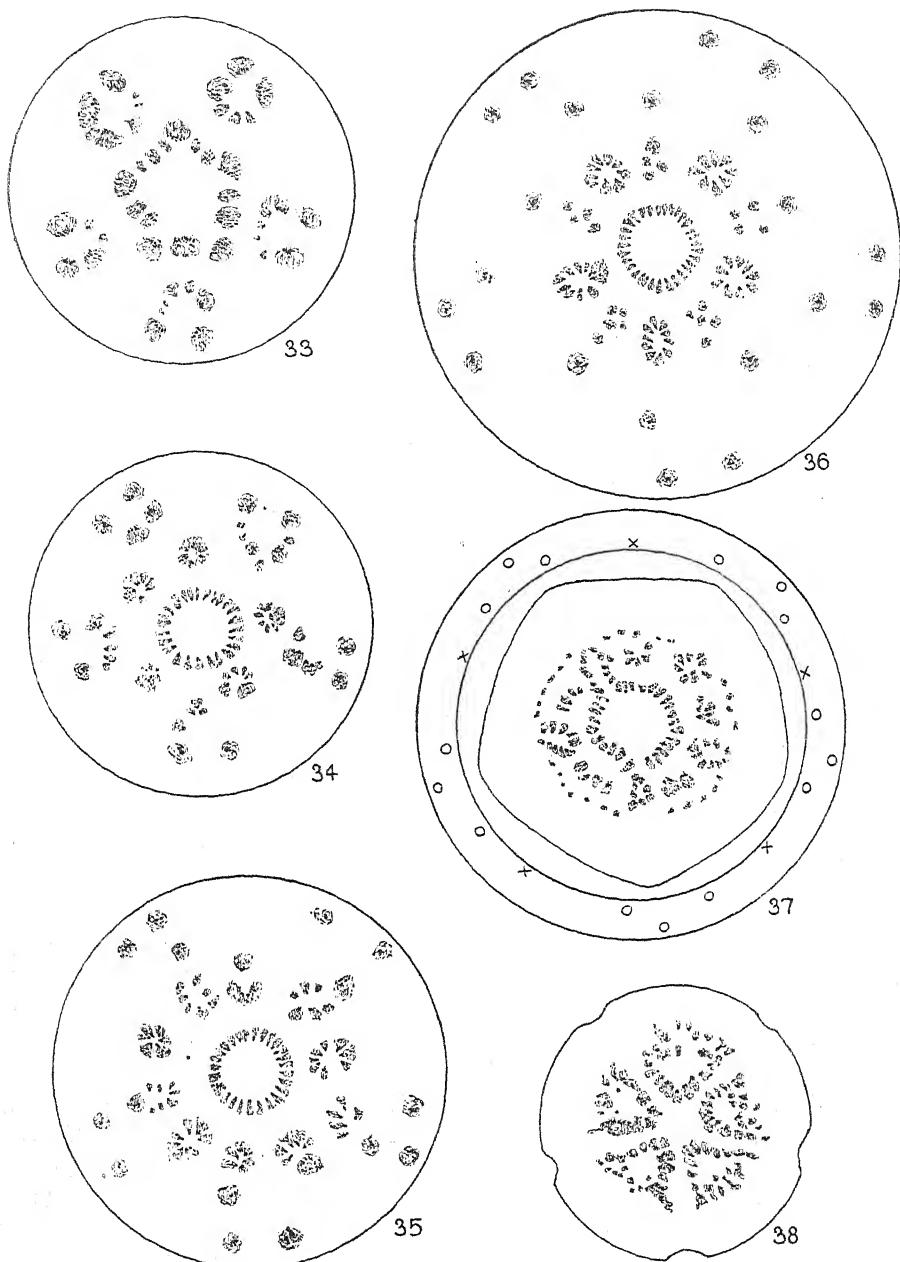
The androecium may be either one-whorled and is then alternitepalous (*Cola*), or two-whorled (*Sterculia*, *Brachychiton*), but in either case all the members produce anthers. When two-whorled the antetepalous members develop first, usually undergo deduplication, and become pushed to the exterior to accommodate the developing ovaries. Those on the alternate radii constitute an inner whorl and remain single. The flower is thus obdiplostemenous in scheme as in the preceding sections, but as there are no actual petals on the petal radii the break in the alternation is shifted to the other set of radii.

The gynoecium is constructed on the same plan as in the preceding sections, consisting of five solid, sterile, and five semi-solid, fertile members, but here, owing to the non-development of the petals as such, sterile cords, loculi and stigma rays stand in line with the tepals. Through the median splitting of the semi-solid members the gynoecium comes earlier or later to consist of five separate ovaries, each composed of $\frac{1}{2}1\frac{1}{2}$ carpel members. In *Cola* the styles are distinct from the outset, but the separation of the ovaries does not occur at once. In *Sterculia* and *Brachychiton* the ovaries separate very early, but remain conjoined by the styles. In a still young *Firmiana* ovary the half carpels open out from one another, exposing the seeds on the margins of the now flat and leaf-like structure. Indeed, so striking is the superficial resemblance to a leaf that *Firmiana* has often been cited as affording indubitable ocular proof that the carpel is simply a modified leaf. We now see that these fertile leaf-like structures composing the *Firmiana* gynoecium are in fact equivalent, not to one single leaf but to one whole leaf conjoined to two half leaves, a construction which explains the otherwise anomalous system of venation, for the lateral veins, which we should expect to take origin from the mid-vein, all spring from the marginal half cords (Fig. 44).

In accordance with the above interpretation the floral formulæ for the different genera are as follows:

Cola acuminata, R.Br., $T5A5_2$ alternitepalous $G5$ antetepalous, sterile + 5 fertile.

Sterculia alata, Roxb., *Brachychiton populneum*, R.Br., $T5A5_2$ (antetepalous) + 5 $G5$ antetepalous, sterile + 5 fertile.



FIGS. 33-8. *Pterospermum acerifolium*, Willd. All from transverse sections taken at successively higher levels. 33. The androgynophore. Towards the outside a ring of five horse-shoe-shaped groups of strands serving the five fertile stamen groups; in the centre a vascular pentagon from which the bundles at the angles will shortly turn outwards to serve the five staminodes. 34. The same after the bundle for each staminode has turned out from the central cylinder and has given rise to a ring-like complex. The horseshoe-shaped group of strands serving each fertile stamen triplet has broken up into four masses, three of which become condensed into the individual stamen cords, while the fourth portion, which occupies a constant position, breaks up

The sterile carpels pushing outwards cause the two members of the antetepalous stamen pair to diverge laterally, so that the two androecium whorls together come to give the appearance of five triplets alternating with the ovaries.

Sterculia (Firmiana) platanifolia, L., T₅ A₅₃ antetepalous + 5 G₅ antetepalous, sterile + 5 fertile.

The thrust of the sterile carpels to the exterior causes the triplet of stamens to diverge, one to one side and two to the other, thus giving later, with the staminodes, an artificial, asymmetrical grouping into quartets alternating with the ovaries.

HELICTEREAE (Figs. 33-42).

Flowers ♂, obdiplostemenous, with androgynophore, calyx and corolla present, antepetalous stamens in groups, loculi antepetalous.

Pterospermum acerifolium, Willd., K₅ C₅ A₅ (staminodes) + 5₃ antepetalous G₅ + 5. We have in *Pterospermum* a typical obdiplostemenous, pentamerous flower with antepetalous staminal triplets and antesepalous staminodes, but the mode of origin of the vascular cords for the several whorls differs somewhat from that characterizing the preceding sections, and allows the steps in the process of deduplication to be easily traced. The emergence from the central ring of the vascular components for the sepals is followed by the turning outwards on the alternate radii of five trunk cords which break up at once into a component for each petal and a residual portion in the form of a ring for the superposed triplet of stamens. The elements forming each of these rings shortly become aggregated into four groups of strands, two and two, one of the two inner groups being weaker than the other three. The three larger groups condense into single bundles which turn outwards to become the cords of the members of an antepetalous stamen triplet; the fourth, weaker set of strands, which in some ancestral form no doubt supplied a fourth, fertile stamen, and so completed the symmetry, fails to become condensed into a single cord, and is discarded. In the meantime, five additional trunk cords have turned out from the central cylinder on the alternate radii. These break up into two portions, one of which is similarly discarded, while the other turns outwards into the staminode. This behaviour indicates that probably in some earlier form the antesepalous members underwent deduplication in

further and is later discarded. 35. The same after complete differentiation of each triplet of fertile stamen cords; the unused portions are seen as ring groups of strands alternating with the ring groups for the staminodes. 36. The same after these latter ring groups have each similarly broken up into a bundle for the staminode on the corresponding radius and a group of discarded strands. 37. The ovary surrounded by the staminal tube, from which it is now disjoined. In the centre the residual vascular ring serving the gynoecium, surrounded by an outer ring of the unused portions originally detailed for the androecium. 38. The same after rearrangement of the whole vascular residuum into five triangular groups of strands standing in line with the petals.

the same manner as those standing in front of the petals. Such deduplication is actually indicated in existing forms in the tri-partite staminodes of *Commersonia* (Buttneriaceae), and occasionally in flowers of *Fremontia* (Fremontieae) (see Fig. 45). As the bundles for the stamens and staminodes pass to the periphery, there remain behind on equidistant radii the ten discarded portions of the trunk cords originally designed for the androecium, and nearer the centre a reformed ring serving the gynoeceum. Above the level of exsertion of stamens and staminodes this central ring breaks up into five sections, which, together with the discarded elements from the androecium, form five triangular groups of strands. These groups break up as follows. Five bundles turn outwards on the radii of the petals and become the sterile carpel cords. The great bulk of the remaining elements continue upwards in the centre as five sets of twin bundles on the radii of the sepals, forming the main cords of the five fertile carpels. A small number of undifferentiated elements wander into the pith, and are discarded. By this time the discarded elements from the staminodal trunk cords have come to an end. Those from each fertile stamen complex group themselves beside the fertile carpel cords, until in turn they also cease. As the ovary enlarges, the sterile carpel members lengthen radially, and are at first continuous with the pith so that the loculus comes into being as a double chamber. As carpel and pith come shortly to be disjointed the loculus becomes a single cavity, but remains partially chambered to the top owing to the plate-like form of the sterile carpel. As the ovary passes into the style column, the twin bundles of the fertile carpels continue upwards as ten separate strands, but the sterile carpel cords are no longer traceable.

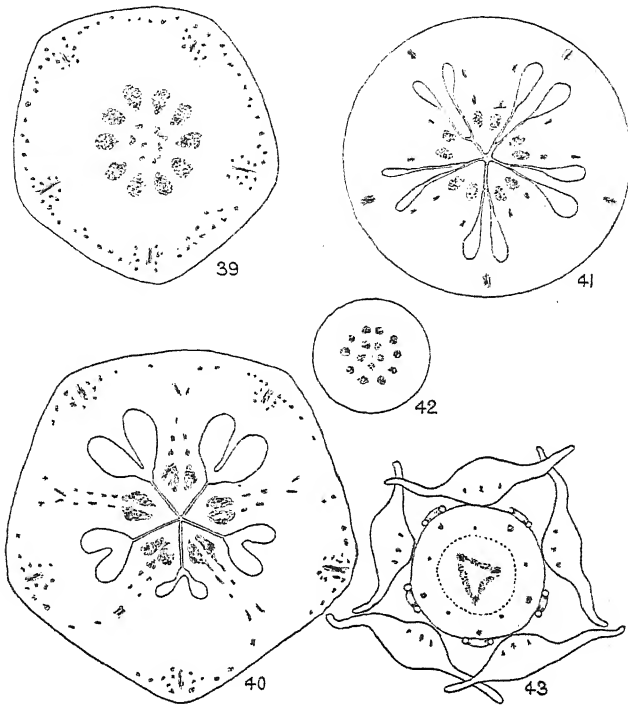
LASIOPETALAE (Fig. 43).

Flowers ♂, obdiplostemenous, calyx and small corolla rudiments present, stamens single, loculi, when 5, in line with the petals.

Thomasia solanacea, J. Gay. In *Thomasia* we have a very interesting stage intermediate between the forms with a fully-developed two-whorled perianth and those with a single whorl of coloured tepals.¹ Here ten cords leave the central cylinder for the perianth as in *Cola*. Five become the midribs of the five outer, coloured, perianth members. The other five on the alternate radii which should correspondingly serve five petals bifurcate, one half entering the perianth member on the right, the other that on the left as in the Sterculieae, leaving very reduced gland-like structures which represent the all but vanished petals without vascular elements. The

¹ In *T. solanacea* the tepals are white with a central pink stripe, but in other species, notably *T. grandiflora*, the likeness of the tepal to a hairy sepal with half a coloured petal tacked on to each side is particularly striking, recalling the appearance of *Sterculia discolor* (see above, p. 100).

flower is evidently well on the way towards completely losing all trace of a corolla as such, and has already converted its sepals into petaloid struc-



FIGS. 39-43. All from transverse sections taken, when in series, at successively higher levels. 39-42. *Pterospermum acerifolium* (continued). 39. The ovary after a bundle from the mid-line of each wedge has turned outwards to become a sterile carpel midrib, leaving behind the two lateral portions so that the residual ring in the centre now shows ten regularly disposed bundles. The elements discarded from the outer ring group of bundles originally appropriated to the androecium now again turn out to the periphery, forming scattered strands which persist for a time, becoming grouped beside the sterile carpel midribs. 40. The same after the appearance of the loculi. The ten bundles of the central residual ring have become differentiated by pairs into the twin bundles of the fertile carpel cords, from which branches pass outwards into the ovary wall. Lines of cleavage extend from the inner angle of each loculus to the centre (the pith having now come to an end) demarcating the inner face of the fertile members. 41. The same showing the partial chambering of the loculi due to the plate-like form of the sterile carpels. The strands in the outer wall of the ovary derived from the ring groups appropriated originally to the androecium have come to an end. 42. The ovary at the level at which it tapers into a style column. In the centre five areas of conducting cells on the radii of the sterile carpel midribs, which can no longer be distinguished; on the intervening radii the twin bundles of the fertile carpel cords. 43. *Thomasia solanacea*, J. Gay. Flower base at the level of exertion of the sepals and gland-like petals. The sepals each show a central and two lateral bundles derived respectively from the whole cords on the same set of radii and a half cord from those on the alternate radii to right and left. Within the outline of the androgynoeium a ring of ten bundles serving the androecium, each bundle having been carried out with, and then disjoined from, one of the original ten outgoing cords for the perianth. The trimerous gynoecium is defined but not yet disjoined from the androecium ring. In the centre the bundles for the three sterile carpels are about to turn outwards from the points of the residual triangle; those for the fertile members are differentiated later from the bundle masses on the three flat sides.

tures. The petal remnants still so far affect the organization of the flower that the obdiplostemonous condition is of the type characteristic of forms

with a double perianth, the fertile stamens standing in line with the petals, the staminodes in line with the tepals. The gynoecium is reduced to three outer, sterile, solid, and three inner, fertile, semi-solid members. The full formula will therefore be T5 C5 minute, without vascular tissue (altogether lacking in some species) A5 (staminodes) + 5 antepetalous G3 + 3.

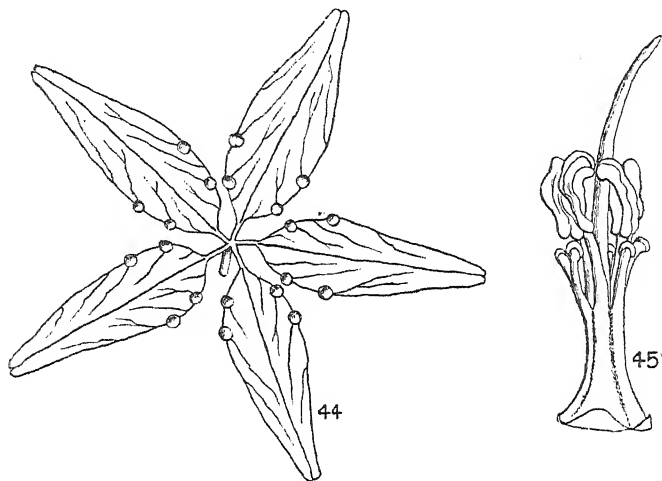


FIG. 44. *Sterculia (Firmiana) platanifolia*, L. Developing fruit after median longitudinal splitting of the fertile carpels so as to produce five separate structures each consisting of $\frac{1}{2}1\frac{1}{2}$ carpels. The secondary veins arise from the half cords of the fertile members. 45. *Fremontia californica*, Torr. Androecium tube surrounding the style column. (From an exceptional specimen with groups of staminodes in addition to the single functional stamens).

FREMONTIEAE (Figs. 17, 18, and 45).

Flowers ♂, perianth in the form of tepals, functional stamens single, staminodes in groups, single, or absent, loculi in line with the tepals.

Fremontia californica, Torr. The perianth consists of five coloured members, united only towards the base with slightly induplicate edges. The three bracts which are present are exerted directly beneath the perianth. The vascular bundles for the androecium leave the central cylinder either with, or immediately after, the cords for the perianth, which itself carries the staminal tube. The circumstance that the cords for bracts, perianth and androecium all emerge in such rapid succession, together with the fact that those for the perianth members break up at once into a number of strands, gives rise to a vascular complex not easy to unravel. But the clue obtained in the study of the Sterculieae enables us to make out the situation. Each perianth segment in *Fremontia* receives not only the vascular complement on its own radius, but also half the complement of the radius on each side. Not only so, but the character of the tissues forming the marginal strips of each segment differs from that in the central portion.

In fact, these coloured segments are evidently tepals comparable with those occurring in the Sterculieae, each being constructed of the tissue material which in some ancestral form served $\frac{1}{2}1\frac{1}{2}$ perianth members. The staminal tube usually carries five anthers, but in one of the flowers examined staminodes with half anthers arising singly or two or three together alternated with the fertile members, which in turn alternated with the tepals. As the vascular strands for all members of the androecium are differentiated from the bundles which have entered the perianth tube earlier, the occurrence of a staminode whorl has no effect upon the position of the carpels. The gynoecium, as in other pentamerous genera, consists of five solid, sterile carpels, which here stand in line with the tepals, and five semi-solid, fertile members on the alternate radii, as in the Sterculieae. As the ovary tapers into the style-column the sterile cords can no longer be traced. The twin bundles of each fertile carpel continue upwards, each pair alternating with the arms of the stylar canal.

The formula for *Fremontia*, in accordance with the above account, will therefore be $T_5 A_{[5_{1-3} \text{ staminodes}]} + 5 G_5$ (sterile, in line with the tepals) + 5 (fertile).

SUMMARY AND CONCLUSIONS.

1. A study of the vascular anatomy of certain sterculiaceous flowers provides a clue to various apparent anomalies in the floral structure, and shows that a consistent principle underlies the variations in position of the several whorls in different genera.

2. In all the genera investigated the syncarpous gynoecium is two-whorled, the outer carpels being solid and sterile, the inner semi-solid and fertile.

3. In Sterculieae certain longitudinal splitting of the semi-solid carpels between the twin bundles of the fertile cord leads to the resolution, while still in the flowering stage, of the syncarpous, pentamerous gynoecium into five separate ovaries. The individual ovary which outwardly has the appearance of being monomerous is in reality constructed of $\frac{1}{2}1\frac{1}{2}$ carpels. This construction accounts for the characteristic venation scheme, which, on the old view that each ovary was formed of a single carpel, remained an unexplained anomaly. Now that the superficial likeness of the prematurely opened, flat, leaf-like ovary of *Sterculia* to a single dorsiventral leaf is shown not to spring from morphological equivalence, the venation scheme presents no difficulty.

4. Genera with the full number of floral whorls are consistently obdi-plostemenous as in Geraniales and Ericales, and from the same cause—carpel polymorphism. When isomerous throughout the sterile carpel

cords, loculi and stigma rays, as well as the fertile stamens, which may be single or in groups, are antepetalous (*Theobroma*, *Dombeya*, *Pterospermum*).

5. In the Sterculiaceae and in *Thomasia* and *Fremontia* where the perianth consists of a single whorl of five coloured members, these members are not homologous with the sepals of a two-whorled perianth. Each represents more than one individual leaf, for each corresponds in its make-up to a sepal bordered on both sides by a half petal. That is to say, each member of the perianth in the above-mentioned forms is equivalent to $\frac{1}{2}1\frac{1}{2}$ members of a normal two-whorled perianth, and may aptly be termed a tepal. Thus in the Sterculiaceae the separate tepal like the separate ovary is a compound structure—the product of two simultaneous and opposite processes in development, fission, and union. This interpretation of the one-whorled petaloid perianth in these types will probably be found applicable to certain cases in other families.

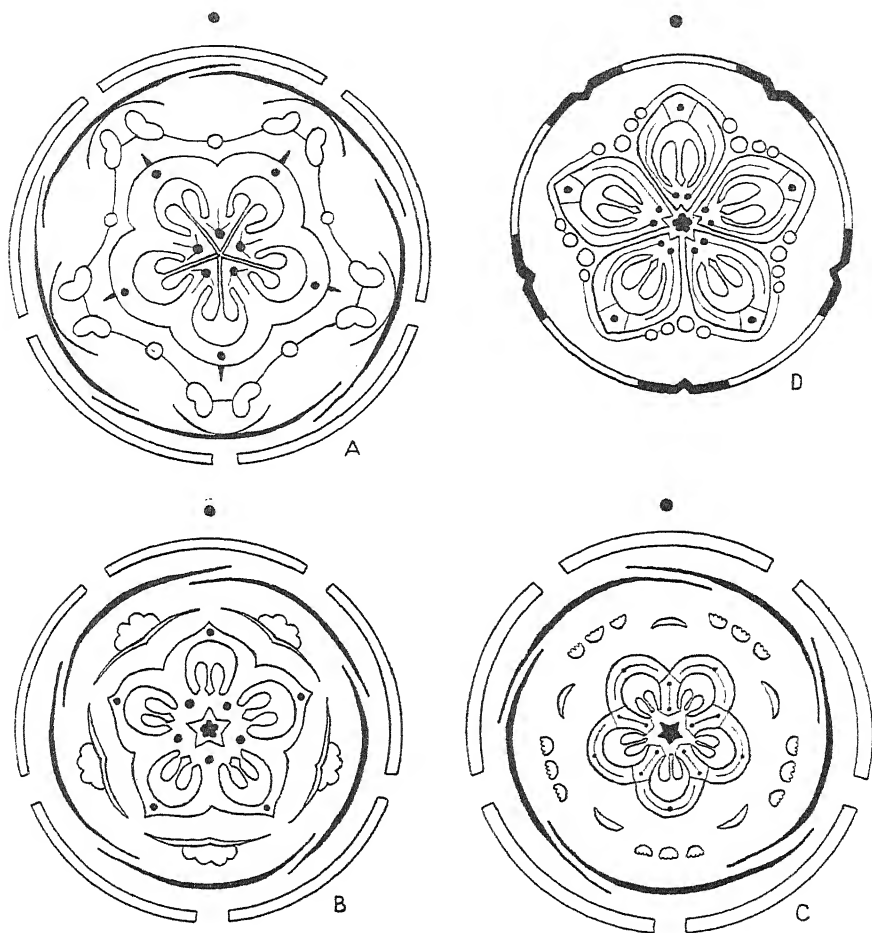
6. In those types having a perianth of tepals and a pentamerous gynoecium the sterile carpel cords, loculi and stigma rays stand in line with the tepals, and the fertile carpels on the alternate radii (Sterculiaceae, *Fremontia*). When both staminal whorls are present and are borne on a gynophore, the outer stamen groups also are antepetalous, the inner single stamens alternitepalous (most Sterculiaceae), but in *Cola* with no second staminal whorl the paired stamens of the outer whorl alternate with the tepals. *Fremontia* differs from the Sterculiaceae in that the vascular bundles for the androecium are differentiated from bundles which have already passed out into the tepals. Here, if an outer whorl of staminodes is present the single or grouped member stands in line with the tepals, the single functional stamens of the inner whorl alternate with them. *Thomasia* presents an intermediate stage in the transition from a perianth of two whorls to one of one whorl. Although the petals, as such, have almost disappeared, and the other members of the perianth are of tepal form, the flower has remained obdiplostemenous.

7. In genera lacking the antesepalous staminodes but having both calyx and corolla, the fertile stamen groups are still superposed on the petals as in the complete flower, but the sterile carpel cords, loculi and stigma rays, as might be expected, stand in these cases in line with the sepals (*Hermannia*).

8. The manner of origin of the vascular cords for the members of the antepetalous stamen groups in the large flower of *Pterospermum* suggests that deduplication in the androecium is brought about not by lateral branching but by successive bifurcation, the odd number of members (often 3) eventually constituting each group being due to subsequent degeneration of one of the division products.

The relations detailed above are illustrated in the accompanying diagrams.

The figures here reproduced were drawn by Miss D. F. M. Pertz to whom I am once more deeply indebted.



- A. *Theobroma Cacao*, L.
 B. *Hermannia plicata*, Ait.
 C. *Dombeya Burgessiae*, Gerr.
 D. *Sterculia (Firmiana) plataniifolia*, L.

I also tender grateful thanks for material to the following : the Director of the Royal Botanic Gardens, Kew, the Directors of the Botanic Gardens at Cambridge, Pisa, and Singapore, the Director of the Department of Agriculture, Peradeniya, Professor T. G. B. Osborn, Professor L. Buscalioni.

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Studies on the Morphology of the Onagraceae.

III. *Taraxia ovata* (Nutt.), Small.

BY

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With Plate VI and four Figures in the Text.

TARAXIA OVATA (Nutt.), Small has been described (1, 3, 8) as exhibiting a wide range of genetic variations, but has never been studied from the standpoint of either genetics or cytology. It possesses many interesting morphological peculiarities. In the present paper it is proposed to discuss megagametogenesis and embryogenesis, to be followed by a discussion of the cytological phenomena in a later paper.

The species has been little studied taxonomically. It was raised to its present position by Small (11), though recently Munz (8), without presenting detailed arguments, reduced *Taraxia* to subgeneric rank. In my opinion, *Taraxia* should retain generic rank, since the morphological differences are alone more than sufficient to distinguish that genus from *Oenothera*.

MATERIAL AND METHODS.

Material for preliminary study was collected late in February of 1927 on a hillside near the southernmost of the Crystal Springs reservoirs in San Mateo County, California. In 1928 a complete series of stages illustrating megasporogenesis and embryogenesis were collected at each of the following stations, all in San Mateo County: the hillside just mentioned; north of Searsville; a railroad cut at South San Francisco; and near Emerald Lake. In all cases except the third, the plants grew only on the southern or south-western sides of the rolling foothills characteristic of the eastern slope of the San Francisco peninsula. Growth began somewhat earlier in 1928 (January 27) by reason of a mild winter and more advanced season and collecting was continued weekly until April 8, when the ground began to dry out. It is remarkable that the roots can survive the long dry season until the winter rains commence at the end of November.

While Petrunkewitsch's fluid was satisfactory for the earlier stages, a modified formalin-acetic-acid-alcohol was better for the later stages in embryogenesis.

To get at the buds and ovaries it is ordinarily necessary to dig up the entire plant and dissect it—a disagreeable task on account of the copious mucilaginous secretion coming from the crown of the rootstock. The necessity of killing the plant to get at the youngest buds is a serious drawback to the availability of this species for cyto-genetical studies.

CHARACTERISTICS OF THE PLANTS.

The species is not widespread over California, but is confined to the foothills within a few miles of the coast, extending from Mendocino County on the north to Monterey County on the south; it is also known from south-western Oregon. Its habitats are sheltered regions of comparative warmth where vernal weather arrives early. The species is often one of the earliest to come to flower; in any event, it is the first of the multitude of coastal Californian onagrads to bloom.

The most characteristic structural feature—and an unusual one for the family—is the thick and fleshy perennial root gorged with small starch grains. The cortex is more or less split and cracked, thus exposing the inner whitish portion. The leaves arise from the crown of the root as a rosette, and the flowers originate in the axils of the leaves, in the normal ratio of about one flower to every ten or twelve leaves, there being extremely wide variation in the proportion of buds and flowers to leaves. The crown of the root is ordinarily below the surface of the ground, the depth varying from 0.8 to 6.0 cm. The leaves are consequently more or less petiolate, those on the outer border of the crown being spread horizontally, whereas the later leaves have shorter petioles and are erect. My plants averaged 15 cm. in breadth; Brandegee's (1) ranged from 10 to 45 cm., and Shinn (10) mentions even larger ones, but I have never observed a specimen (including offshoots) that exceeded 22 cm. Even plants brought into the garden and heavily fertilized did not show an appreciable increase in size.

METHOD OF PROPAGATION.

There is no record of this plant ever having been raised from seed. Dr. L. L. Burlingame repeatedly self- and cross-fertilized plants growing in the experiment garden, but failed to obtain seedlings when the seeds were sown. Brandegee (1) figures a 'young plant showing cotyledons and first leaf', but outside of the spread of the seeds by torrential rains says nothing regarding its propagation. Some observers suggest that ground squirrels and other burrowing rodents, which abound in *Taraxia* localities,

transport portions of the rootstocks to new regions.¹ My own observations indicate that the species is increased by a purely vegetative method, namely, the splitting of the crown, with the formation of offshoots which grow slightly above and away from the parent root, and eventually become completely detached. The process begins immediately after growth recommences during early winter; during the flowering season one or more offshoots can ordinarily be found on every large plant, but no offshoots are developed during the dry season. Many isolated patches are apparently of such clonal origin. The species, owing to a variety of causes, appears to be becoming extinct.

Seeds collected early in 1928 were sown in the winter of the same year. The germination percentage was fifty-eight, but not one seedling developed a plumule, and all soon perished. The seedlings, being hypogeal, exhibited only two cotyledons, an elongated hypocotyl and a slightly swollen primary root. As will presently be described, the embryo fails entirely to develop an apical meristem of any nature.

THE 'FILIFORM CALYX-TUBE'.

That part of the flower extending from the apex of the ovule-containing portion of the ovary to the adnate disc of the hypanthium had been described (9) as the calyx-tube, and this erroneous conception persisted for over eighty-six years. The broadly obconic calyx-tube, based on measurements of a large number of living specimens, averages 2 mm. The question concerning the morphology of this so-called 'calyx-tube' appears to have been settled, but I believe that further study is required. The conception that it is 'a sterile portion of the ovary' seems doubtful, for there is no substantial evidence that at some earlier period in the phylogeny of the species it contained ovules which subsequently disappeared. It is more reasonable to conceive of its development as an adaptation (the elongation of the apex of the ovary) to carry the flowers above the leaves, since otherwise they would remain hidden among the latter.

Curran (3) states that the 'calyx-tube' of *Taraxia* is very similar in structure to that of *Gongylocarpus*, 'and throws some light upon the peculiar method of fruit formation in that genus'. However that may be, *Gongylocarpus* is certainly far removed phylogenetically from *Taraxia*, and bears a closer affinity to *Stenosiphon* than perhaps to any other onagrad.

¹ Grinnell and Dixon (Monthly Bull. Calif. State Hort. Comm., vii, nos. 11-12, p. 597-708) state that none of these animals are known to eat the rootstocks of any plant; the ground squirrel found in San Mateo County is a pronounced spermophile.

THE OVARY.

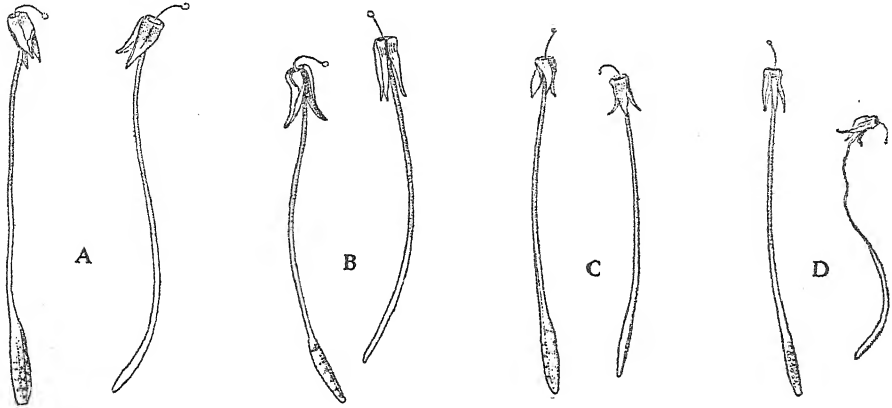
Not every ovary is fertile (i. e. in the sense that it contains ovules): variable percentages of the total number of ovaries are entirely destitute of ovules (Text-fig. 1, A-D). Twenty plants were dug at random in the Searsville locality, and the flowers were classified. The computations follow:

Plant.	Capsules.		Fully-opened Flowers.		Older Buds almost open	Younger Buds.	Sterile Buds.
	With Ovules.	Sterile.	With Ovules.	Sterile.			
I.	10	6	2	3	2	3	6
II.	5	3	4	3	6	22	—
III.	9	4	1	3	2	7	3
IV.	6	4	3	3	1	10	2
V.	9	3	3	2	3	16	3
VI.	10	3	5	3	2	28	—
VII.	10	7	3	1	1	7	7
VIII.	16	6	3	3	5	24	—
IX.	3	1	2	—	1	—	—
X.	10	1	4	3	2	19	—
XI.	6	3	3	2	4	23	2
XII.	8	1	4	2	2	3	2
XIII.	3	4	3	2	2	5	—
XIV.	2	6	1	2	1	1	3
XV.	5	19	—	1	3	3	14
XVI.	7	9	—	—	—	—	4
XVII.	19	4	2	1	—	3	11
XVIII.	4	8	3	—	1	2	5
XIX.	7	3	4	2	5	19	—
XX.	5	13	—	—	—	—	6
Averages	8	5	3	2	2	10	3
%	23.54	16.51	7.64	5.50	6.57	29.81	10.39

It will be noticed that in some plants (e. g., plants XV and XX) there are more sterile capsules than fertile, and in about as many instances (e. g., plants XV and XVII) the sterile young buds outnumber the fertile. Estimations on plants from South San Francisco gave practically the same values, expressed in percentages. In the figure (Text-fig. 1, A-D) the difference between a wholly sterile ovary and one containing ovules is clearly perceivable. The sterile ovules soon disintegrate in the mucilaginous fluid which surrounds them, and it is consequently easy to overlook those hidden in the axils of the leaves towards the periphery of the rootstock.

There are no definite locules in mature capsules. These exist only in the younger ovaries; at about the time the megagametophytes approach maturity, there occurs an extensive hypertrophy of the ovarian tissues bordering the locules, with the result that in maturing capsules the locules are obliterated, and the two rows of anatropous ovules are apparently

distributed irregularly throughout the mass of an almost solid ovary. In the vertical plane, the ovules are arranged alternately to the right and left of a median line. Three or four ovules in each ovary are oriented in the reverse of the usual anatropous position, but this is not such a consistently



TEXT-FIG. 1, A-D. Fertile (at left) and sterile ovaries. Each set from the same plant, and drawn shortly after the petals dropped. From the following localities: (A) Experiment garden; (B) Crystal Springs; (C) Searsville; (D) South San Francisco. Drawn natural size and reduced $\frac{1}{2}$ in reproduction.

regular feature as it is in some onagrats, e. g., *Anogra trichocalyx*. The average number of ovules to each ovary is twenty-six. The ovules, owing to absence of regularity in arrangement, and because of the ingrowing ovarian walls, are of varying sizes. The larger ovules are, so far as I have observed in over 125 onagrats, the largest in the family; their size is not due to increase in cell size, but in the total number of cells plus an unusually thick and apparently abnormal seed coat.

The capsules are of very irregular shape when approaching maturity, generally becoming acute at each end, flattened on opposite sides, and are usually sessile, although Brandege found that they are sometimes 'narrow or linear, and occasionally more or less pedicellate'. The walls are thin, chartaceous, and conform to the bulging ovules, which at first are purplish, but become yellowish-white after the development of the unusual seed coat. The capsules are generally indehiscent.

THE OVULE.

The origin and structure of the young ovule is remarkably uniform throughout the Onagraceae, and there were no observed departures from the typical in *Taraxia ovata*. Outside of mentioning that the inner integument and megasporocyte originate simultaneously, and that the outer integument appears later and at the same time that the ovule begins to curve downward, it is unnecessary to describe in detail the development and structure of the various ovular organs.

THE MEGASPOROCYTE.

The megasporocyte arises in exactly the same manner as has been described for *Hartmannia tetraptera* (6). In *T. ovata* the number of tapetal layers is considerably greater, but this does not appear to be a matter of consequence in so far as the entrance of the pollen tube is concerned. In only one case has more than one megasporocyte in the same ovule been observed (Pl. VI, Fig. 1).

THE QUARTET.

It is unfortunately necessary to omit discussion of details regarding the two meiotic mitoses from which originate the linear row of four megaspores; a great quantity of material was collected, but on account of the high percentage of wholly sterile ovaries a complete series of stages was not secured. However, it may be said that the mode of meiosis is telosynaptic and the haploid number of chromosomes is seven. The micro-pylar megaspore is always the functional one and the two mitoses therein are regular (Pl. VI, Figs. 2, 3).

THE MEGAGAMETOPHYTE.

The organization of the megagametophyte proceeds as has been described for *Hartmannia tetraptera* (6), but irregularities are markedly prevalent. It is particularly interesting to note that the departures from the typical found in *Clarkia elegans*, *Hartmannia tetraptera* and *Taraxia ovata* are not similar, but are peculiarly characteristic of each species.

T. ovata is characterized by the excessive liability of the megagametophyte cytoplasm to shrink, and at the same time to stain so intensely with the usual chromatin stains that little can be made of its structural details. This is especially true of the synergids, and often becomes noticeable even before these two cells have acquired definite organization (Pl. VI, Figs. 5-7). In the illustrations it is impossible to convey an adequate representation of the appearance of this condition.

The cells comprising the hypostase are excessively safranophilic, at times becoming red-black, but the megagametophyte, perhaps because of the lesser density of its cytoplasm, does not reach this extreme.

In the normal mature megagametophyte (Pl. VI, Fig. 4), as contrasted with that of other onagrads, the synergids are elongated, the egg cell is smaller and contains a smaller vacuole, and a nucleus very deficient in chromatin, and the polar nucleus is, as in *Hartmannia tetraptera*, inclined towards the non-nucleolate condition.

When the functioning megaspore has attained the limit of longitudinal extension, it has broadened but little from the original width of the mega-

sporocyte; just as the second mitosis in the embryo sac begins, a peculiar degeneration of the neighbouring nucellar cells becomes evident (Pl. VI, Fig. 4, 10). This condition is prevalent in other onagrad, but is extreme in *T. ovata*, except in embryo sacs whose contents have early degenerated. It always originates at the outer periphery of the embryo sac, immediately adjacent to the position occupied by the egg-cell, and rapidly advances.

A typical representative of the normal megagametophyte is portrayed in Pl. VI, Fig. 4. In the type most commonly observed the synergids are degenerated and the upper part of the egg-cell gaps widely (Pl. VI, Figs. 5, 6). The filiform apparatus is rarely absent, but the synergid indentations generally are. In the common, intensely stained megagametophyte few or no starch grains are present in the cytoplasm, but they are conspicuously abundant in perfectly normal sacs. The question therefore arises: Are these starch grains present at the expense of the nucellus, and are they an indication of normality? Apparently they are in both cases.

FERTILIZATION.

The ovule is not oriented, before fertilization, in the vertical plane, but at an oblique angle. The microgametophyte upon entering the embryo sac tends to travel in a strictly vertical direction, consequently the egg-cell is usually twisted away from its normal position. This explains why nearly all young embryos seem to be attached to the nucellus at one side, instead of being located in a line with the tapetal layers and the micropyle.

Syngamy proceeds essentially as described for *Clarkia elegans* (7), save that the nucleoli of the fused gametic nuclei remain distinct for but a very short time. The union of the secondary male nucleus with the polar nucleus was not followed out since the young 'endosperm' nowhere exhibited any nuclear irregularities. At the time of fertilization all the participating nuclei appear to be very weak in chromatin, though the nucleoli are comparatively large in size.

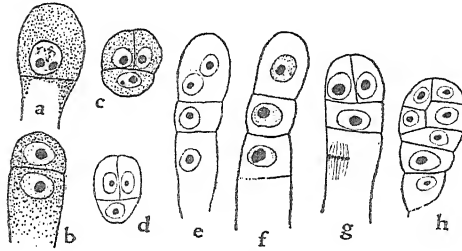
EMBRYOGENESIS.

The zygote presents nothing out of the ordinary.

The stages in embryogenesis, which are uniform for the family, so far as known, have been fully described in the second paper of the present series (7), to which reference should be made.

The primary wall erected following the first zygotic mitosis is transverse (Text-fig. 2, *b*). Sometimes the nucleus of the primary suspensor cell is missing (Text-fig. 2, *a*); although no binucleate unicellular embryos similar to those described for *C. elegans* (7, Figs. 2, 3) have been observed,

a later three-celled stage, of which the apical cell is binucleate, has been found more than once (Text-fig. 2, *e*). A trinucleate unicellular embryo may occasionally be encountered. Three- to five-celled filamentous embryos are frequent (Text-fig. 2, *e, f*); they are, to judge from still older stages, normal (Text-fig. 2, *h*) and distinct from apogamous filamentous structures (compare with Text-fig. 3, *a*).



TEXT-FIG. 2, A-H. Early embryogenesis. Explanations in text. The mitotic figure in *g* has fourteen chromosomes on each spindle. $\times 475$.

The divisions are normal up to the time that the peculiar concave wall in the hypophysis is about to be formed (Pl. VI, Fig. 12). A not inconsiderable portion of the blame for the upsets in the rhythm of divisions in the young embryo may be charged to irregularities in the appearance and position of this peculiar cell-wall. It may fail to appear (Pl. VI, Figs. 13, 16); it may be unduly delayed; the vertical wall may be formed precociously, so that the wall arises in 'sections' as an oblique wall in each of the four adjoining daughter cells (Pl. VI, Fig. 17); as a whole, the hypophysis cell exhibits a marked independence of other groups of cells in the young embryo. Despite juvenile irregularities, the hypophysis cell (or the group of daughter cells) possesses a strange ability invariably to fulfill its function of developing the root tip and root cap, as will presently be demonstrated.

Morphogenesis in the onograd embryo is essentially a rhythmic process and requires, from the quadrant stage onward, the most perfect co-operation among the various constituent groups of cells as a prerequisite to the successful culmination of the series of events in a perfect, normally constituted new individual. Once the cadence is interrupted, after first becoming established, the balance is never recovered and a monstrosity results. Embryonic structures which are of 'illegitimate' origin, such as the apogamous embryos to be described presently, are characterized by the absence of a rhythmic succession of purposeful cell divisions: such structures might be called the result of the lack of the formative and directive influence of morphogenetic factors brought into being as one result of fertilization.

As noted in a preceding paragraph, the stage figured in Pl. VI, Fig. 12, represents the end of growth according to the normal scheme for the Onograceae. To describe the irregularities which subsequently appear, one

may discuss two alternatives, but it is difficult to designate the one to which the greater importance should be ascribed. One is that growth (in bulk) in the four upper central cells of Pl. VI, Fig. 12, is retarded; the other is that development in the lower group of four cells is normal and uninfluenced by inhibitive factors. An examination of Pl. VI, Fig. 15, brings this point out sharply; Fig. 13 is an intermediate stage, but this particular embryo is dubiously representative because of its hypertrophied epidermal cells. In Fig. 15 the lower three tiers of cells are perfectly regular in all respects, but the arrangement of the upper groups is chaotic and the cells are reduced in size to half or less. The nuclei of these upper cells are small and markedly deficient in chromatin, hence one might visualize a condition in which chromatin insufficiently underlies morphogenetic imperfections.

It will be noted that the cells are normal towards the periphery of the globular embryos (Pl. VI, Figs. 14-20); in other words, those cells giving rise to the cotyledons are unaffected. However, the growth and development of the cotyledons is irregular, the amount and nature of the irregularity depending upon the extent to which the remainder of the embryo is influenced by nuclear deficiency (compare Pl. VI, Figs. 23-5).

It requires some caution in stating whether the root develops normally or precociously, for there is nothing in this species upon which to base comparisons. Comparison with other species (e. g. *Godetia amoena*) indicates precocity, or at least an unusual degree of rapidity in growth. It will be noticed in Pl. VI, Fig. 24, that the radicle has far outstripped the cotyledons in development.

Pl. VI, Figs. 23-5 illustrate, in outline, three embryos of approximately the same age, each from a different locality, and the differences in the extent of radicle development are striking. In Fig. 23 the vascular supply to the cotyledons has already been organized, while it is doubtful if any vascular tissue would ever be developed in the type represented by Fig. 24.

Sometimes no embryonic organs except the cotyledons are ever developed (Pl. VI, Fig. 22). In the centre, immediately below the epidermal layer, are about three tiers of horizontally flattened cells from which by no stretch of the imagination can apical meristematic tissues be expected to originate. In somewhat similar embryos the cells just below this group of flattened cells displayed an organization highly suggestive of a radicle with two oppositely placed root caps, situated at right angles to the normal position; i. e., in a horizontal plane and passing parallel to and between the cotyledons.

While it is probably true that no definite, unvarying correlation between number of chromosomes and morphology can be postulated, it nevertheless remains that variations from the constant number of chromo-

somes may profoundly affect the normal morphology of the species or hybrid (e.g., mutant forms of *Oenothera Lamarckiana*). If these variations are to become effective factors in morphogenesis in the higher plants, they must of necessity initiate activity quite early in embryogenesis; though these factors may remain latent until the octant stage, it is doubtful if they would be able to exercise any appreciable influence on the development of the embryo itself if delayed beyond the early formative stages. It proved extremely difficult to find satisfactory mitoses in the embryos under discussion, but I am convinced that the trouble does not primarily lie in variations in the number of chromosomes. All the figures displayed fourteen diploid elements, which number is considered constant for *T. ovata*. Meiosis in the megasporocytes of this plant apparently has not been affected by the evident general degenerance of the species as a whole.

If one accepts the existence of growth-regulators (or, as they are variously called, growth-hormones, growth-accelerating or retarding substances, &c.), difficulties are immediately encountered when considered in connexion with the embryos of *T. ovata*. In this plant it becomes necessary at the outset to postulate the existence of specialized growth regulators; that is to say, of separate types which regulate development of the cotyledons, of the radicle, &c. Furthermore, we would need to consider the probable relationship between chromatin behaviour (e.g., as in the present case, chromatin deficiency) and the action of growth regulators.

APOGAMOUS EMBRYOS.

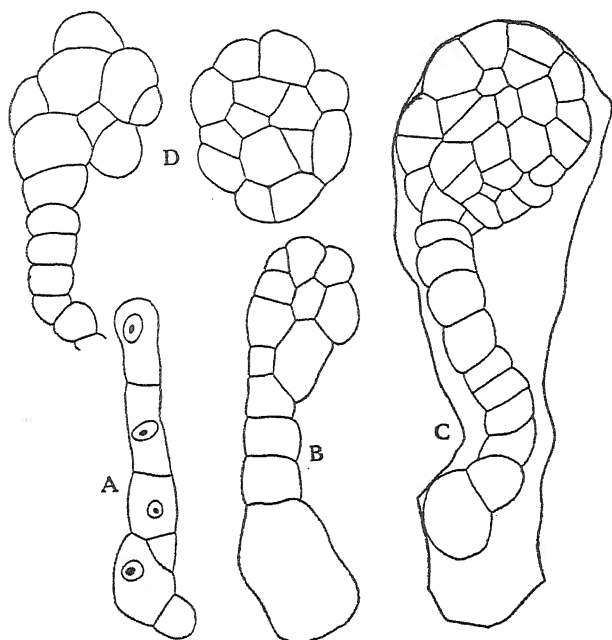
Apogamous embryos essentially similar to those previously described in *Clarkia elegans* (7) occur in *T. ovata*. The principal difference between the structures in the two species is that those in *T. ovata* are of a more filamentous nature (Text-fig. 3, a). At times the filament comprises a linear row of from ten to fourteen cells before a head-like growth occurs at the micropylar end. Occasionally an incipient head may be formed at the opposite end.

No mitosis could be found, hence the chromosomal constitution of the embryos remains unknown. Their apogamous nature is conjectured, first, upon the entire absence of 'endosperm' and second, upon the lack of evidence of the entrance of a pollen tube. Perhaps the filamentous part may represent the growth in length of the quartet rather than the failure of the megasporocyte to undergo meiosis, the latter being manifestly the case in *C. elegans*. If this be true, the structures are therefore haploid.

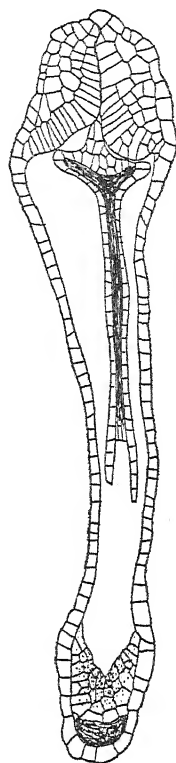
STERILE OVULES.

In addition to ovarian sterility, there are three distinct types of ovular sterility in *T. ovata*. Any or all of the three types may be found in almost every ovary.

(a) That type of sterile ovule common in *Clarkia*, *Oenothera*, *Eucharidium*, and *Godetia*, initiated by the collapse of the nucellar cells on that side of the ovule opposite to the raphe, occurs rarely, perhaps because



TEXT-FIG. 3.



TEXT-FIG. 4.

TEXT-FIG. 3, A-D. Apogamous embryos. A, early stage, which indicates that origin may be from the quartet; the 'head' develops from the micropylar megaspore. B, later stage. C, oldest stage found. The limits of the sac are outlined. D, two sections of the same embryo (22 microns apart) of an intermediate stage. $\times 635$.

TEXT-FIG. 4. Type of sterile ovule in which the nucellus is entirely eliminated. $\times 240$.

of the stronger turgor and thicker walls of the nucellar cells. When this type of sterility does appear, all ovules in the ovary are affected.

(b, c) These two types have several characters in common: nucellar cells are almost entirely absent; the integuments are each only one layer of cells in thickness and never develop the typical lignified structure of the mature seed coat: the chalazal region is fairly well developed, but no hypostase appears. In the first of these two types there is an entire absence of sporogenous tissue (Text-fig. 4), whilst in the other sporogenous tissue was originally present, and distinctly apogamous embryos are often to be found. The difference is therefore resolved into one of the time at which collapse occurred. The cause appears to lie in physiological disturbances

at an early stage in the development of the ovule, which principally affect the nucellar cells. If the disorders occur before the archesporial initial is formed type *b* results; if after, type *c*.

ENDOSPERM.

The endosperm, whose nuclei are diploid as regards chromosomal content, is mainly collected at the chalazal end of the embryo sac, sometimes becoming quite dense. The nuclei are usually bi- or trinucleolate. At the micropylar end of the sac there is only a very small quantity of plasm of a 'stringy' nature. Elsewhere the plasm ranges from stringy to highly vacuolate with the beginning of cell formation evident.

During early embryogenesis the hitherto coenocytic endosperm cytoplasm undergoes a change simulative of cell formation, which is accomplished by the segregation of small masses of plasm around a single uni- to multinucleolate nucleus. These units are physiological rather than morphological, for this is not a true process of cell formation but is merely an early phase in the eventual degeneration and disappearance of the 'endosperm'.

SUMMARY.

1. *Taraxia ovata*, still characteristic of the Californian endemic flora, is now becoming extinct, owing principally to its lost ability to reproduce by seed.
2. The species produces a high percentage of buds entirely destitute of sporogenous elements.
3. The true calyx-tube is broadly obconic and averages 2 mm. in length. The elongated apex of the ovary is an adaptation to carry the flowers above the leaves.
4. The ovules are probably the largest in the Onagraceae.
5. The mode of meiosis in the megasporocyte is telosynaptic. The haploid number of chromosomes is seven, the diploid fourteen.
6. The micropylar megaspore of the linear quartet is invariably functional and the organization of the megagametophyte proceeds normally.
7. The megagametophyte is characterized by an excessive tendency to shrink and to stain intensely with the usual chromatin stains.
8. Fertilization is normal; no chromosomal disturbances were observed.
9. Early stages in embryogenesis are normal, but at about the time of differentiation in the hypophysis cell a peculiar method is thenceforth followed. The final result is an embryo possessing only two cotyledons, a short hypocotyl and radicle.

10. Morphogenesis in the onagrad embryo is essentially a rhythmic process requiring the utmost co-ordination among the various constituent groups of cells if the series of events is to culminate in a perfectly balanced new individual. Monstrosities result from interruptions in cadence.

11. Apogamous embryos, frequently observed, probably result from the continued growth of all four megaspores of the quartet.

This study was prosecuted during the tenure of a National Research Council Fellowship in the Biological Sciences, with residence for part of the time at the New York Botanical Garden.

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EXPLANATION OF PLATE VI.

Illustrating Mr. Donald A. Johansen's Paper on Studies on The Morphology of the Onagraceae.

All Figures $\times 950$, reduced $\frac{1}{2}$ in reproduction.

Fig. 1. Two megasporocytes, the micropylar (lower) apparently functional.

Fig. 2. Functioning megaspore; three lower megaspores of the quartet degenerating.

Fig. 3. Binucleate megagametophyte.

Fig. 4. Normal megagametophyte—rarely encountered. Notice presence and distribution of starch grains. Polar nucleus is non-nucleolate.

Figs. 5, 6. The type of megagametophyte usually observed. Synergids unorganized and highly safranophilic. Observe that the egg cells are open above.

Fig. 7. Abnormal megagametophyte; apparently a single 'synergid' and a false egg-cell.

Fig. 8. Binucleate megagametophyte (?) degenerating. Nucleolus of lower nucleus is amoeboid.

Fig. 9. Outline of highly safranophilic megagametophyte. One synergid, egg-cell, and polar nucleus. The identity of the other free nucleus is doubtful; perhaps it may represent the nucleus of the missing synergid.

Fig. 10. Outline of longitudinally constricted megagametophyte. The synergids are well indented, but each lacks a basal vacuole. The egg-cell is the only completely enclosed one I have ever observed in any onagrad.

Fig. 11. Abnormal embryo sac.

Magnifications as noted, reduced $\frac{1}{2}$ in reproduction.

Figs. 12-19. Embryogenesis. $\times 950$.

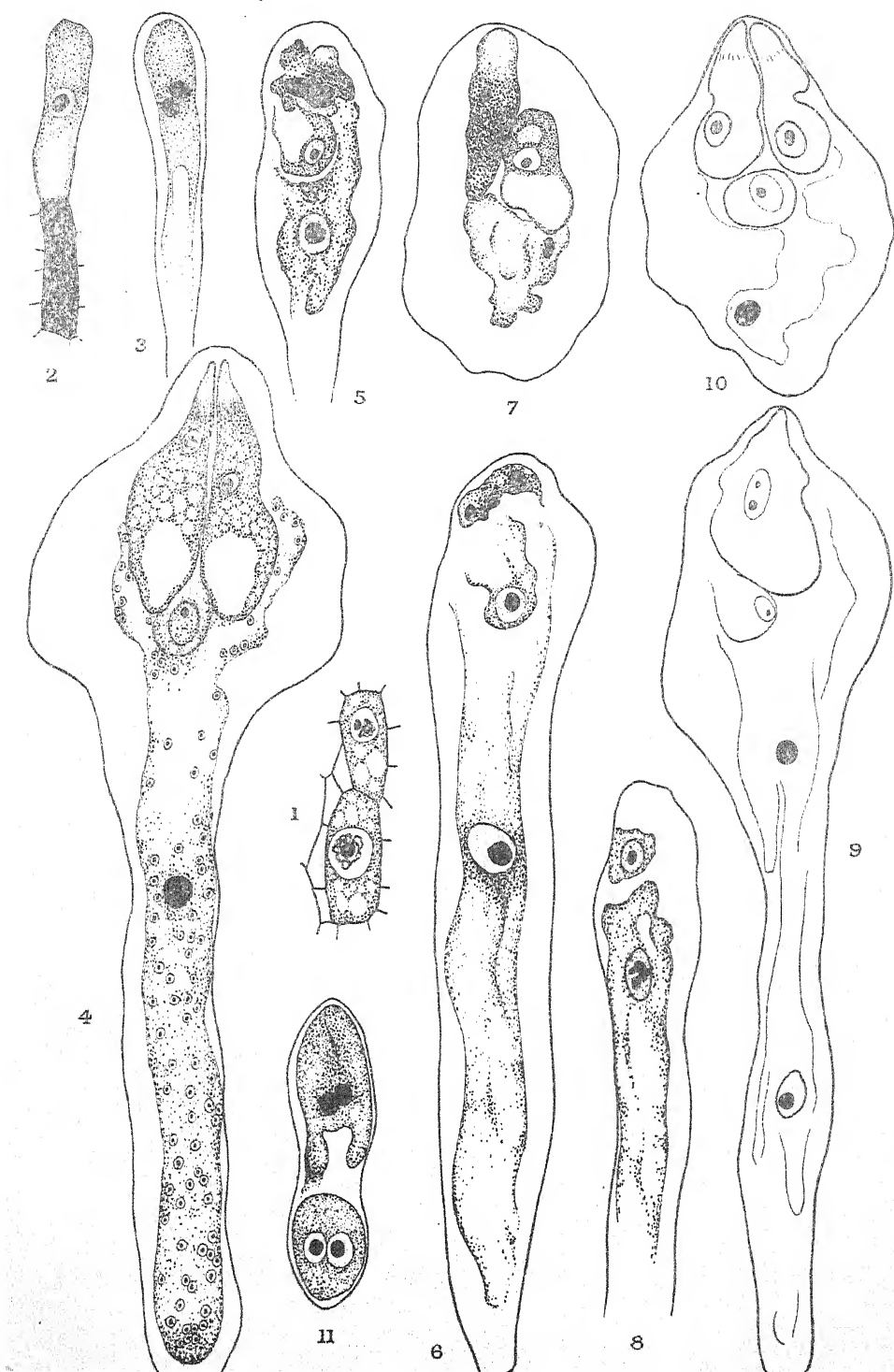
Fig. 20. Radicle already definite. $\times 950$.

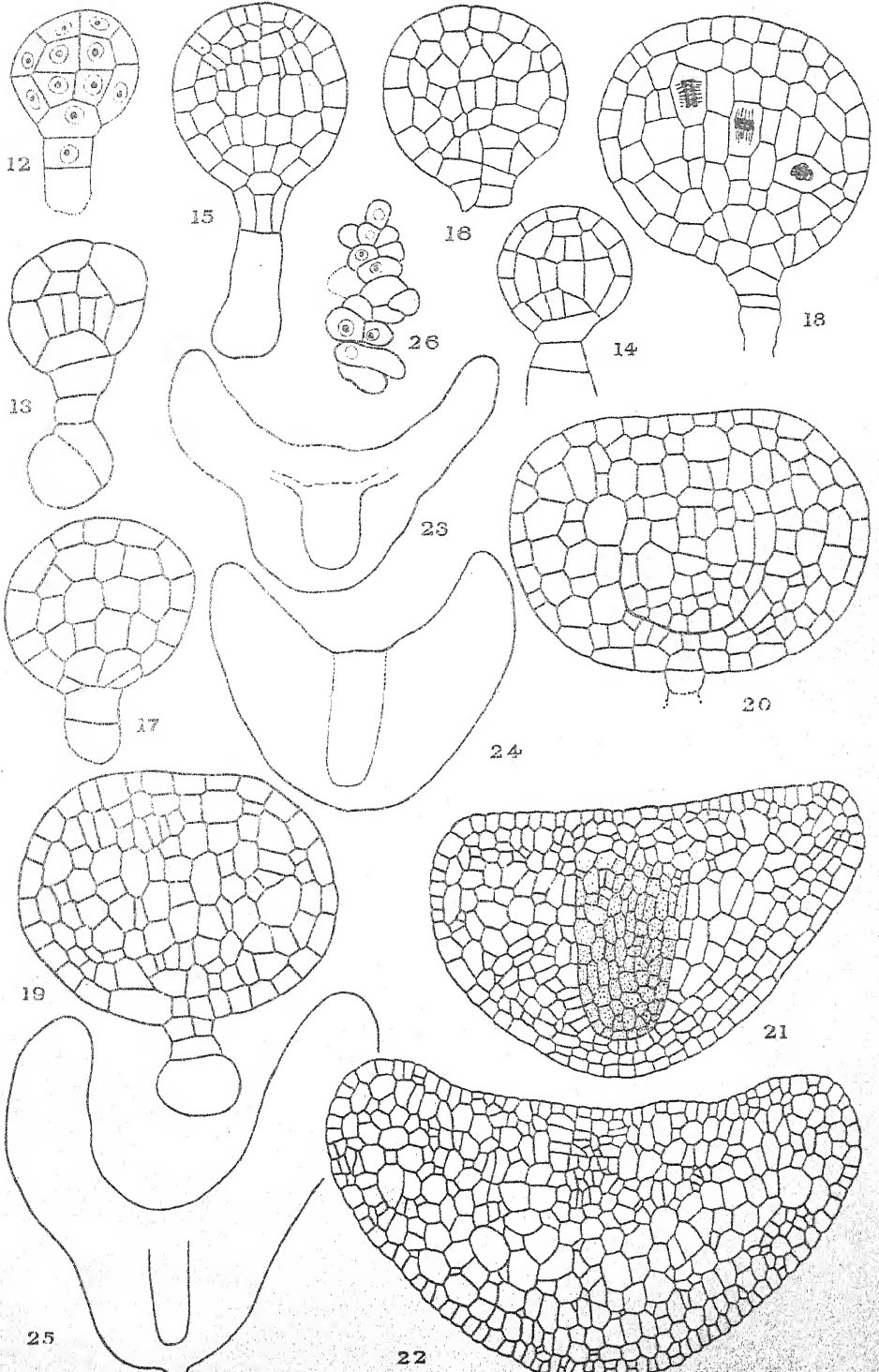
Fig. 21. Later stage; beginning of cotyledon development. $\times 450$.

Fig. 22. Radicle absent. $\times 450$.

Figs. 23-5. Development of cotyledons (in outline); all approximately identical developmental stage. $\times 240$.

Fig. 26. Monstrosity of doubtful origin. Endosperm is present in the embryo sac. $\times 950$.





Further Studies on Transport in the Cotton Plant.

I. Preliminary Observations on the Transport of Phosphorus, Potassium, and Calcium.¹

BY

T. G. MASON

AND

E. J. MASKELL.

With four Figures in the Text.

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¹ Paper No. 8 from the Physiological Department of the Cotton Research Station, Trinidad.

SECTION I. INTRODUCTION.

INVESTIGATIONS on the transport of carbohydrates (23) and of nitrogen (18–22) in the cotton plant have led us to certain conclusions which may be summarized as follows. Organic nitrogen compounds as well as carbohydrates are, in the main, synthesized in the leaf and are exported from the leaf via the phloem to other parts of the plant. When the plant is in active (vegetative) growth there is, in consequence, a downward movement of carbohydrates and of nitrogen along the bark of the main axis towards the roots. If this movement is interrupted, by removal of a ring of bark from the main axis between the foliage region and the ground, carbohydrates and nitrogen diminish in bark and wood below the ring but accumulate in bark and wood above the ring and also in the leaves. If only a partial ring is made the total rate of downward movement is diminished, but the rate across unit cross-sectional area in the constricted region is increased. The direction of movement depends on the relative positions of ‘source’ (foliage region) and ‘sink’ (leafless regions of the plant), and the normal downward movement in the bark of the main axis may experimentally be reversed by reversing the relative positions of the leafy and the leafless portions of the stem.

These observations, and the fact that the diurnal fluctuations in concentration of sugars and of nitrogen in the sap of the leaves are reflected, with a time lag of a few hours, in the sap of the bark tissues, suggested that translocation via the phloem might be determined by concentration gradients of the mobile forms of carbohydrates and of nitrogenous compounds. Examination of the vertical gradients in the bark revealed a fairly simple relation between rate of movement of carbohydrate and the gradient of sugar concentration. We were led, therefore, to explore the possibility that a similar relation between gradient and movement held for nitrogenous compounds, and in general for all materials that are translocated via the phloem. On this view the function of the phloem would be the acceleration of the diffusive movement set up, for each mobile substance, between the region of supply and the region of utilization.

This formulation of the characteristics of phloem transport deliberately leaves open the nature of the mechanism responsible for acceleration, whether protoplasmic streaming, as Curtis (7) has recently maintained, or some as yet unknown mechanism.¹ For our present purpose we wish

¹ Recent microscopic observations at this station on the phloem of cotton have shown, in agreement with the observations of Curtis (7) and others on other plants, that protoplasmic streaming (up to 3 cm. per hour) regularly occurs in the young sieve-tubes near the cambium, and also, throughout the phloem, in the elongated phloem parenchyma cells which run parallel with the sieve-tubes and, in cotton, comprise nearly half the ‘sieve-tube groups’. We have not, however, observed any streaming in mature sieve-tubes with open pores. If streaming is restricted to phloem parenchyma cells and the young sieve-tubes it would appear, from the cell dimensions, that the maximum possible acceleration of diffusive movement, assuming diffusion across the cell-walls at the

merely to note that this view implies *independent* movement, between source and sink, of the different materials that are mobile in the phloem, the rate and direction of movement of each being determined by the concentration gradient of each. In opposition to this general picture there is the theory, recently urged by Münch (26), that translocation in the phloem is a pressure movement of total cell sap in the direction of the total osmotic pressure gradient, the differences in osmotic concentration being due to assimilation or solution of reserves on the one hand, and use or storage of assimilates on the other. Now although, in all our experiments with cotton, nitrogen and carbohydrate were both moving via the phloem in the same direction, i. e. away from the foliage region, there did appear to be considerable independence in the rates of movement of the two materials (18, 22). The simplest proof of independence would, however, be furnished by a case in which one had movement of two different materials in opposite directions along the same region of phloem. If Curtis's conclusions (6, 7, 8) are correct, such a case is provided by the downward movement of carbohydrates on the one hand and the upward movement of nitrogen and of ash constituents absorbed by the roots on the other, both the upward and the downward movement taking place in the phloem.

In the case of the cotton plant, however, the results we obtained on ringing showed that the upward transport of nitrogen absorbed by the roots of the cotton plant takes place mainly via the wood, presumably in the transpiration current. It would appear that, in virtue of its great transpiration pull, the leaf obtains not only the bulk of the water but also the bulk of the nitrogen absorbed by the roots. Also, when we consider the low absolute concentration of nitrogen in the tracheal sap, it is clear that the leaf is in fact concerned with concentrating the absorbed nitrogen compounds as well as with transforming them chemically.

This result for one of the elements absorbed from the soil raises the question whether the other soil nutrients show similar transport phenomena. Does upward transport take place with the transpiration current in the wood, or is it mainly via the bark? If upward transport is through the wood vessels, does the bulk of the material absorbed go, along with the water, to the leaf; or is it in the main abstracted by the surrounding tissues during the upward transport? If the bulk of the material goes to the leaf, is it merely concentrated, as it must be, owing to the transpiration loss, or is it also chemically altered; and, in either case, to what extent is it re-exported from the leaf to the other parts of the plant?

same rate as in water, is of the order of 1,000. We should require, however (cf. 23), an acceleration of more than 20,000. Consequently, until we can obtain evidence of streaming within mature sieve-tubes and also in and out of the sieve-pores, it seems that the question of mechanism must be left open. Meanwhile, we may attempt, by observations on movement of materials and on longitudinal gradients, to establish whether the kind of mechanism required is in fact of this type, i. e. an acceleration of diffusive spread.

These questions concern what we may call the immediate fate of the mineral material absorbed by the root. There is the further problem of the mobilization and redistribution of mineral elements temporarily stored in certain parts of the plant. In cotton, as in many annual plants (cf. 30), the greater part of the absorption of mineral elements takes place during the earlier part of the plant's life, before fruiting begins. Thus, by the time half the dry weight is attained, the cotton plant has already taken up 80-90 per cent. of the total amounts found in the mature plants of the elements N., P., S., K., Ca, and Mg. (cf. 5). During the later stages of growth there must, therefore, be a redistribution of materials from the older regions to the growing points and the developing fruits. By what channel is this redistribution effected, and what determines the direction of movement? How, for example, is the 'hunger' of a growing tissue for phosphorus or for potassium satisfied? In the case of the nitrogen re-exported from the leaf via the phloem we have adduced evidence supporting the view that transport is determined by the concentration gradient which is set up between the region of utilization and the region of supply. Does this apply to distribution of other mineral elements? Does the phloem, by means of its mechanism for accelerating diffusion, form the necessary link between tissues containing stored material of any kind and tissues needing that material for growth, or are there materials for which this link is not available?

Most discussion on the transport of mineral elements has centred round the role played by transpiration. In view of recent work such as that of Hasselbring (15) and of Muenscher (25), the older idea of a simple relation between transpiration rate and rate of salt uptake cannot be maintained. Although, in all Muenscher's experiments, when the transpiration rate was increased total ash content increased to a somewhat greater extent than did the dry weight, this increase was, except where light intensity was varied, very much smaller than the increase in transpiration rate. Curtis (8) points out that, if salts are translocated upwards in the transpiration current, increased transpiration rates should tend to increase uptake indirectly, by accelerating the removal of salts within the plant from the absorbing region. He has accordingly questioned whether the transpiration current plays any part in transferring soil nutrients from the root to the foliage region and, further, on the basis of some ringing experiments (6) has concluded that supply via the wood only is inadequate to maintain the normal content of ash and nitrogen. As noted earlier, the nitrogen results we have obtained in ringing experiments with cotton are the direct reverse of those of Curtis. We suggested (18) that the divergence might be due to the very much longer interval elapsing between ringing and sampling in Curtis's case. Other points of difference in method may be noted. In our experiments the main axis was ringed; the stem below

the ring was thus completely isolated from the foliage region except via the wood. With this arrangement we ran the risk of starving the root of sugars and so diminishing salt uptake; but in an experiment lasting usually less than two days it seemed likely that this risk could be neglected. In so far as this moderate starvation did diminish salt uptake, the observed accumulation of nitrogen in the leaves and stem above the ring becomes still more significant.

In Curtis's experiments (6), using a perennial plant, 'there were always normal branches below the ringed one, so that the roots and trunk below would be well supplied with food and their absorptive ability would not be lost through death or starvation'. With an arrangement of this kind the tissues below a ring are in complete organic connexion with foliage that is drawing its supply of water through uninjured wood. The foliage above the ring, on the other hand, must draw its supply of water and salts *past* the ring and must, moreover, do this in competition with the foliage on uninjured branches. In view of these disadvantages, the positive evidence, which as we showed (18) Curtis's own experiment provides, of the ability of leaves to draw nitrogen via the wood past the region of a ring, becomes very significant. In this experiment of Curtis's data were not obtained for ash content. In his other ringing experiments, where data exist for the initial ash content, the mean increase in ash content above a ring was 19 per cent. as against 73 per cent. in the normal branch. Clearly some upward transport had taken place via the wood, and Curtis's conclusion that supply via the wood is *inadequate* to maintain normal content could only be accepted if it were quite clear that there had been no impairment of the transpiration current. The long time interval makes this extremely doubtful.

More recently Curtis (7) has shown that chilling the stems of Bean seedlings interferes 'with the upward movement of materials very much as it interferes with the backward movement of sugars', and interprets this as supporting the theory 'that the upward translocation of solutes takes place chiefly through the phloem'. If, however, we examine his figures for the water and ash content of the tops of the seedlings (i.e. above the chilled region) we find that the uptake of ash in the chilled set is as much as 60 per cent. of the uptake in the normal set. Moreover, the net increase in water shown by the tops in the chilled set is only 59.4 per cent. of that in the normal set or, in other words, ash uptake and net water uptake are equally affected by the chilling. When we note also that the viscosity of water at 20°C. (the room temperature was 20°–23°C.) is only 65.5 per cent. of that at 5°C. (the bean stems were chilled to 2°–5°C.) it is difficult to see why the results were not interpreted as being due to an effect of chilling on the upward movement of the tracheal sap. The results would seem, in fact, to strengthen the view that inorganic nutrients move up with the transpiration stream.

In view of the clear-cut results as to the channels of nitrogen transport in the work on cotton, it was decided to make determinations also of certain ash constituents. These observations, made on the dry material collected originally for the nitrogen work, are intended as a survey, preliminary to more elaborate work in which a larger number of individual elements will be estimated. One great advantage of using this material was that the transport of the ash constituents could be compared with that of nitrogen and of carbohydrates: also that, so far as nitrogen and, probably therefore, other mineral nutrients were concerned, the plants were known to be in the stage of active uptake from the soil. It could thus be fairly assumed that we should be dealing with the primary transport distribution of substances absorbed by the roots. It was recognized that the behaviour of individual elements would be of particular interest, and though, on account of the limitation of material, only three elements could be estimated, these were chosen so as to throw some light on the differences that might exist. Of the three, namely, phosphorus, potassium, and calcium, phosphorus is absorbed as, and largely exists in the plant as, an anion, but enters also into the molecular constitution of a number of organic compounds including proteins. Potassium and calcium are monovalent and bivalent cations respectively, occupy widely different positions in the lyotropic series, are very different in the solubilities of their salts, and have been considered also to differ in their mobility within the plant.

The experiments to be considered deal with the effect of ringing and with some aspects of the problem of uptake by the boll.

SECTION II. METHODS.

(a) *Material and Analyses.*

The methods employed in drawing samples, in handling the material and in calculating the sampling errors, have already been described (18–23). The methods of analysis used for carbohydrates and for nitrogen have also been described (18–23). Briefly these were as follows:—(1) total sugars were estimated, from analyses made on the expressed sap, as total-reducing sugars after invertase inversion; (2) polysaccharides were estimated as total-reducing sugars after acid hydrolysis of the dried material from which the sugar had first been leached by 10 per cent. alcohol; (3) total nitrogen was estimated on the dried material by the Kjeldahl method, modified to include nitrates. For the *ash determinations*¹ 3–6 grm. of the dried material was moistened with sulphuric acid and ashed at a dull red heat in a muffle furnace to constant weight. The ash was taken up in hydrochloric

¹ We have to thank Professor F. Hardy and Mr. R. R. Follett-Smith of the Chemical Department, Imperial College of Tropical Agriculture, for information as to the details of the analytical procedures.

acid, the volume made up to 250 c.c., and phosphorus,¹ potassium, and calcium were determined on aliquots of the solution. Phosphorus was precipitated from the aliquot as ammonium phosphomolybdate and estimated by titration of the precipitate with standard alkali. Potassium was precipitated as sodium potassium cobaltinitrite and estimated by titration of the precipitate with standard permanganate. The standard permanganate was calibrated, for each set of ash determinations, by means of parallel determinations, using the same technique, on a range of KCl solutions of known concentration. Calcium was precipitated from the faintly acid aliquot by ammonium oxalate, and the resulting precipitate of calcium oxalate titrated, after solution in acid, with standard permanganate. As in the case of nitrogen the results are given as mg. of the elements. Total ash is given as mg. of the sulphated ash.

(b) *Calculations.*

In calculating, from the amounts determined per grm. dry weight, the amounts present in specified regions of the plant at different times or after different treatments, we have followed the procedure described in earlier papers (18-23). In short-period experiments (lasting 1 to 2 days), with fairly mature tissues, we have shown that the residual dry weight (dry weight less (sugars + polysaccharides)) not only varies much less than fresh weight or dry weight, but shows as a rule no significant variation in time or as a result of treatment. The mean residual dry weight for each specified region is accordingly taken as the basis for expression of results. In experiments lasting two days or more, and in the case of rapidly growing organs such as young bolls, the residual dry weight shows an appreciable increase in time, and the results must be expressed as weight per sample (per plant or per boll).

(c) *Comparisons between ash constituents and other substances.*

In short-period experiments when, as noted above, residual dry weight is practically constant, increment of carbohydrate is represented with sufficient accuracy by the increment of *labile carbohydrates* (total sugars + polysaccharides). In longer experiments much of the carbohydrate imported is transformed to structural material, and there is consequently a measurable increase in residual dry weight. Confining attention to labile carbohydrates would then underestimate the amount of carbohydrate imported. In earlier papers (20, 22) we have, in such cases, used the change in dry weight less 5.7 times the weight of nitrogen as an estimate of the change in carbohydrate material. Since we now have data for ash, it

¹ The phosphorus determined on the sulphated ash may show a slight loss (about 4 per cent. in the bark) when compared with determinations made on material ashed at low heat with magnesium nitrate.

seems advisable to allow for this fraction as well, and in this paper *total carbohydrate* will be taken as (dry weight—5.7 N-ash). We do not imply that all the material included is carbohydrate, but that it is mainly of carbohydrate origin. There will be some loss in weight due to condensation of the imported carbohydrate, but the relatively small correction that would be required is not known for our conditions, and for the purposes of the present study the calculation adopted appears a reasonable estimate.

SECTION III. THE EFFECTS OF RINGING.

(a) *Introduction.*

In this section three experiments will be discussed, all carried out on plants that were kept in a vegetative condition by flower-bud pruning. In the first two we study the effect of removing a ring of bark from the main axis between the foliage region and the root. In one of these experiments the first collection was made $5\frac{1}{2}$ hours and the last 24 hours after ringing, the mean time being $13\frac{1}{2}$ hours. In the other, the collections were made on the day after ringing, the mean time being 30 hours. The third experiment was a study of the possibility of reversing the normal downward movement, along the main axis, of substances exported from the leaves. The collections were made two days after ringing, the mean time being 52 hours. All the experiments are relatively short from the point of view of interference with the transpiration current by blocking of the wood, or of interference with uptake by starving the root. In the third, however, the time was long enough to allow of a definite increase in residual dry weight.

With regard to the interpretation of results there are three possibilities for each of the mineral nutrients. (1) Accumulation below and decrease above the ring will indicate upward movement via the bark. (2) Absence of any appreciable difference, either above or below the ring, between ringed and normal stems will indicate that there is, under the experimental conditions, no appreciable upward movement in the bark, but that the substances are moving upward via the wood and thence spreading radially to all tissues. (When the unavoidable injury to the wood has had time to affect the transpiration current, the ringed stem may lag behind the normal both above and below the ring.) (3) Accumulation above and decrease below the ring will suggest that the material is, like nitrogen, moving up in the wood, passing mainly to the leaves, and from thence is being re-exported via the bark, i.e. that there is a downward movement along the bark of the main axis towards the roots. Alternatively, it is possible that a substance might have accumulated above a ring, not because it was moving downwards in the bark, but because other substances, e.g. carbohydrates, were moving down, and by their accumulation above the ring caused an

increased uptake direct from the transpiration current of the material in question. Similarly the decrease below the ring might be due to the liberation of this substance into the transpiration current as the carbohydrates moved away. It seems probable, however, that, where there has been no tissue growth or increase in residual dry weight, this ought not to obtrude itself as an important factor. For nitrogen we have already other evidence that the first explanation is correct. The case of the other soil nutrients can be more conveniently discussed at a later stage. Meanwhile, in order to simplify the discussion, we shall provisionally accept accumulation above and decrease below a ring as indicating downward movement via the bark. It should be noted that both explanations definitely imply upward movement via the wood.

(b) *Changes above and below a ring.*

(1) *Experiment 2.*¹

Procedure. In this experiment (cf. 21), carried out on October 6 and 7, 1927, the plants were thirteen weeks old and were without flowers or bolls. The stem below the first (i. e. lowest) fruiting-branch was without leaves and branches. The plants thus consisted of a leafless zone of main stem, about 30 cm. long, between the ground and the lowest fruiting-branch, and above this the foliage region formed by the main axis with its leaves and leafy fruiting-branches. There were two groups of plants. In the Normal group, the plants were marked with tape just below the lowest fruiting-branch, while from the plants of the Ringed group a ring of bark was removed at this same level. Two regions of stem were taken for analysis. The Upper consisted of a 20 cm. length immediately above the ring or tape and the Lower of a similar length immediately below the ring or tape. In the laboratory the stem samples were trimmed to a length of 18 cm., the portions abutting on the ring or tape being rejected. One leaf was taken from the main axis of each plant, from the region between the seventh and tenth nodes from the apex. Two samples, each of forty plants, were taken from each group at each collection. The time-table shows the sequence of events.

Time-table.

Oct. 6,	5.30 a.m.	Ringling of Ringed group and initial collection from Normal or Unringed group.
„	„ 11.0 a.m.	} Three collections from Normal and from Ringed groups.
„	„ 4.30 p.m.	
„	7, 5.30 a.m.	

Results. Owing to the short time intervals employed it is not possible to follow with sufficient accuracy the time sequence of changes following

¹ The numbers assigned to the experiments are those used in the series of papers on transport of nitrogen (19-22).

ringing. Attention will be confined, therefore, to a comparison of the mean weights obtained, for Normal and Ringed groups respectively, for the three collections, 11.0 a.m., 4.30 p.m., and 5.30 a.m. The residual dry weight showed no significant effect of time or treatment; accordingly the results are expressed on the basis of the mean residual dry weights for the whole series. The weights of materials in the two regions of stem (bark + wood) are given in Table I. The distribution of the response as between bark and wood will be considered at a later stage (p. 144).

TABLE I.¹

Weights per 18 cm. of Stem (Bark + Wood) above (Upper region) and below (Lower region) first fruiting-branch in Normal and in Ringed groups.
Upper region.

	Normal.	Ringed.	Difference. Ringd minus Normal.	Significant Difference. P = 0.05. P = 0.10.
Labile carbohydrates grm.	1.675	1.858	+ 0.183	0.068
Nitrogen mg.	95.6	100.5	+ 4.9	4.78
Ash	425.6	437.4	+ 11.8	21.5 17.1
Phosphorus mg.	30.76	32.84	+ 2.08	2.28 1.81
Potassium	97.50	98.95	+ 1.45	7.06 5.61
Calcium	43.20	40.40	- 2.80	3.13 2.51

Lower region.

	Normal.	Ringed.	Difference. Ringd minus Normal.	Significant Difference. P = 0.05. P = 0.10.
Labile carbohydrates grm.	2.255	1.900	- 0.355	0.079
Nitrogen mg.	119.1	110.5	- 8.6	4.84
Ash	622.3	592.5	- 29.8	8.9
Phosphorus mg.	39.35	38.70	- 0.65	0.95 0.76
Potassium	138.40	132.25	- 6.15	6.19 4.91
Calcium	59.98	57.05	- 2.93	1.29

It will be seen that there is no evidence of any accumulation below the ring in the Ringed group, but that, on the contrary, all substances decrease. Again, above the ring all substances increase, with the exception of calcium, which decreases both above and below. Thus there is no sign of upward movement via the bark of any of the substances considered. Upward movement must be taking place via the wood. Above the ring the increases of carbohydrate and of nitrogen are quite significant and that of phosphorus partially so. Below the ring the decreases of carbohydrate, nitrogen, and ash are significant, and that of potassium partially significant.

¹ In this and in all subsequent tables statistically significant values ($P < 0.05$) are in heavy type, and partially significant values ($P > 0.05 < 0.10$) in ordinary type, while values which do not reach even the lower level of significance are in italic type.

As the data stand they suggest a downward movement in the bark, not only of carbohydrate and nitrogen, but also of ash, phosphorus, and potassium.

A more accurate test of this response, indicating downward movement, may be made by combining the results for Upper and Lower regions. The excess of the Ringed over the Normal group in the Upper region is added to the excess of the Normal over the Ringed group in the Lower region, to obtain a figure representing the total amount of downward movement into the Upper and out of the Lower regions of the stem. The results are given in Table II *a*, while Table II *b* gives the results of making the same calculations on the sample basis instead of on the mean residual dry weight basis.

TABLE II.
Amounts moved into Upper and out of Lower Regions of Stem.

	(a)			(b) ¹		
	Mean Residual Dry Weight Basis.			Sample Basis.		
	Total amount in Movement.	Significant Value. P = 0.05. P = 0.10.		Total amount in Movement.	Significant Value. P = 0.05 P = 0.10.	
Labile carbo- hydrates grm.	+ 0.538	0.104		+ 0.513	0.166	
Nitrogen mg.	+ 13.5	6.8		+ 12.0	5.1	
Ash "	+ 41.6	23.25		+ 32.3	39.1	30.5
Phosphorus mg.	+ 2.73	2.47		+ 3.50	3.25	
Potassium "	+ 7.6	9.39	7.46	+ 6.03	10.7	8.4
Calcium "	+ 0.13	3.39	2.71	- 1.99	5.40	4.20

It will be seen, Table II *a*, that the values recorded for ash and for phosphorus are quite significant, while that for potassium is partially so. An apparent movement of potassium in the observed direction would have occurred by chance in less than 5 per cent. of cases. Very much the same results are obtained from the calculations made on the sample basis (Table II *b*). The standard deviations are, however, in most cases somewhat greater, and the responses of ash and of potassium appear less significant. Taking the two tables together, downward movement of phosphorus as well as of carbohydrate and of nitrogen is definitely established, while downward movement of ash and potassium appears very probable. Calcium, on the other hand, shows no evidence of either upward or downward movement via the bark.

In Table II *a* we record a downward movement of 41.6 mg. ash. The question arises as to how far this is due to phosphorus and potassium, for the contribution of calcium is negligible. Since we are dealing with

¹ If in Table II *b* we calculate the amount of carbohydrate moved from the values for total carbohydrate (i. e. dry weight - 5.7 N-ash) we obtain the figure + 0.406.

sulphated ash, we will assume that the phosphorus was present as $K_2P_2O_7$ and the excess potassium as K_2SO_4 . On this basis we can account for 20.6 mg. of ash, i. e. about half the amount recorded as in downward movement. There is a suggestion, therefore, of downward movement of ash constituents other than phosphorus and potassium.

In the case of materials exported from the leaves via the bark and moving down the main axis towards the roots, we expect to find an accumulation, not only in the stem, but also in the leaves above the ring. We have shown earlier that this is the case for carbohydrates and for nitrogen. In Table III the values for the ash constituents as well as for nitrogen and carbohydrates in the leaves are given. As in Table I, the results are calculated on the basis of the mean residual dry weight for the three collections of Normal and Ringed groups after the initial collection.

TABLE III.

Total Amounts per Leaf in Normal and Ringed Groups.

	Normal.	Ringed.	Excess of Ringed over Normal.	Significant Excess P = 0.05.
Labile carbohydrates grm.	0.447	0.599	+ 0.152	0.029
Nitrogen mg. . .	108.9	112.8	+ 3.9	3.05
Ash " . .	361.3	378.3	+ 17.0	14.8
Phosphorus mg. . .	16.76	17.18	+ 0.42	0.80
Potassium " . .	55.55	56.53	+ 0.98	2.84
Calcium " . .	71.28	72.93	+ 1.65	3.02

All the substances considered show an increase in the Ringed group. From the significant value obtained for the response of total ash, it is clear that some ash constituents, as well as carbohydrate and nitrogen, have accumulated in the leaves above the ring. To what elements the accumulation is due is not clear, for the responses of the three elements considered, though positive, are not individually significant.

That there should be no loss, but rather an increase, of all the ash constituents in the leaves above the ring, makes it impossible to explain the accumulation in the stem above the ring by a migration out of the leaves, without a corresponding import into the leaves via the wood. Taking the results for leaves and stem together, upward movement of ash, phosphorus, and potassium via the wood appears definitely established, while re-export from the leaves and downward movement via the bark of phosphorus, potassium, and possibly some other ash constituents, appears highly probable. For calcium, on the other hand, there is no suggestion either of upward movement via the bark, or of re-export from the leaves and downward movement via the bark. There is, of course, the possibility that there was no appreciable uptake of calcium by the roots during the experiment. This cannot, unfortunately, be tested on the present data,

since the differences between successive collections are only of the order of the sampling errors. There is the further complication that, whereas the leaves of the ringed plants show a slight increase in calcium content above the normal, the stems show both below and above the ring a significant or partially significant decrease. The interpretation of this apparent inconsistency is not clear.

(2) *Experiment 3.*

In this experiment (cf. 21), instead of determining the changes in Upper and Lower regions of stem, i. e. both above and below a ring on the same stem, only one region of stem was used. In one group of plants, High-ring group, a ring of bark was removed just *above* this region of stem; in the other, Low-ring group, a ring of bark was removed just *below* the specified region of stem. The total response to ringing is measured by the difference between the Low-ring and High-ring groups, an excess in the Low-ring group indicating downward movement and an excess in the High-ring group indicating upward movement via the bark.

Procedure. The plants were sixteen and a half weeks old and were without flowers or bolls. The stem below the first (lowest) fruiting-branch was kept bared of leaves and branches. In the High-ring group the stem was ringed immediately below the first fruiting-branch, and marked with tape 20 cm. below the ring. In the Low-ring group the stem was marked with tape immediately below the first fruiting-branch, and ringed 20 cm. below the tape. In collecting the material the stems were cut at the tape and the ring, and the samples were trimmed in the laboratory to a length of 18 cm., measured from the lower end. For each group there were five samples, each of forty-eight plants. The time-table of operations was as follows:

Oct. 31, 1927.	7.0 a.m.-9 a.m.	Ringling.
Nov. 1, 1927.	12.30 p.m.	Collection of 1st and 2nd samples from each group.
" "	2.15 p.m.	Collection of 3rd sample from each group.
" "	3.30 p.m.	Collection of 4th and 5th samples from each group.

Results. As in the previous paper dealing with this experiment the weights are calculated on the mean residual dry weight basis (Table IV *a*). This, if anything, slightly underestimates the excess of materials in the Low-ring group, since the residual dry weight was slightly, though not significantly, greater in the Low-ring group (cf. 21). For comparison the differences between the groups are shown also as calculated from the actual weights per sample (Table IV *b*, p. 141).

On either basis the Low-ring group shows a significant excess of phosphorus and of potassium, in addition to carbohydrates and nitrogen. The results support the conclusions from the previous experiment and may be regarded as establishing a downward movement, via the bark, of

phosphorus and of potassium. The response of total ash is in the same direction as in the previous experiment, but is not here significant. Calcium alone shows a decrease above the ring, but the small difference between the two groups is not significant and, as in the previous experiment, there is no evidence of either upward or downward movement in the bark of this element. It would appear that the calcium in the stem is mainly derived directly from the ascending transpiration current, and that very little of the calcium reaching the leaf is re-exported to the stem. Once again, however, we cannot altogether exclude the possibility that calcium uptake from the soil had ceased.

(c) *Reversal of Direction of Movement in the Bark (Experiment 9).*

This experiment was undertaken primarily in order to test whether the normal downward movement of nitrogen could, like the parallel movement of carbohydrates, be reversed by reversing the relative positions of the leafy and the leafless regions of stem, and in particular to determine what changes of the concentration gradients in the bark accompanied this reversal. As already described (20), clear evidence was obtained for reversal of movement, accompanied by reversal of the dynamic gradient in the bark. In view of the evidence, from the two experiments just described, that there is normally a downward movement in the bark of phosphorus and potassium as well as of nitrogen and carbohydrate, it was of great interest to examine whether the reversal held for ash materials as well. Since only the dried material was available the question of changes in sap gradients of the ash constituents could not be profitably examined. We confined ourselves therefore to an examination of the changes in total content that occurred in the leafless regions of stem which were acting as the receiving regions during the experiment.

Procedure. Two contiguous regions of stem were chosen, the Upper including five nodes and the Lower seven (cf. Fig. 1). Samples were drawn for three groups of plants. On the day the experiment began the stem was ringed immediately above the Upper and immediately below the Lower region in all three groups. Upper and Lower regions were thus isolated from the rest of the plant, so far as transport via the bark was concerned. In the Leaves-high group all leaves were now removed from the main axis and branches of the Lower region. In the Leaves-low group all leaves were similarly removed from the Upper region. In the Ringed group leaves were removed from both regions, and an additional ring was made between Upper and Lower regions. Thus the Lower region of stem in the Ringed group serves as a base line for the measurement of downward movement via the bark into the corresponding region of the Leaves-high group. The Upper region of stem in the Ringed group

serves similarly as a base line for the measurement of any upward movement into the Upper region of the Leaves-low group.

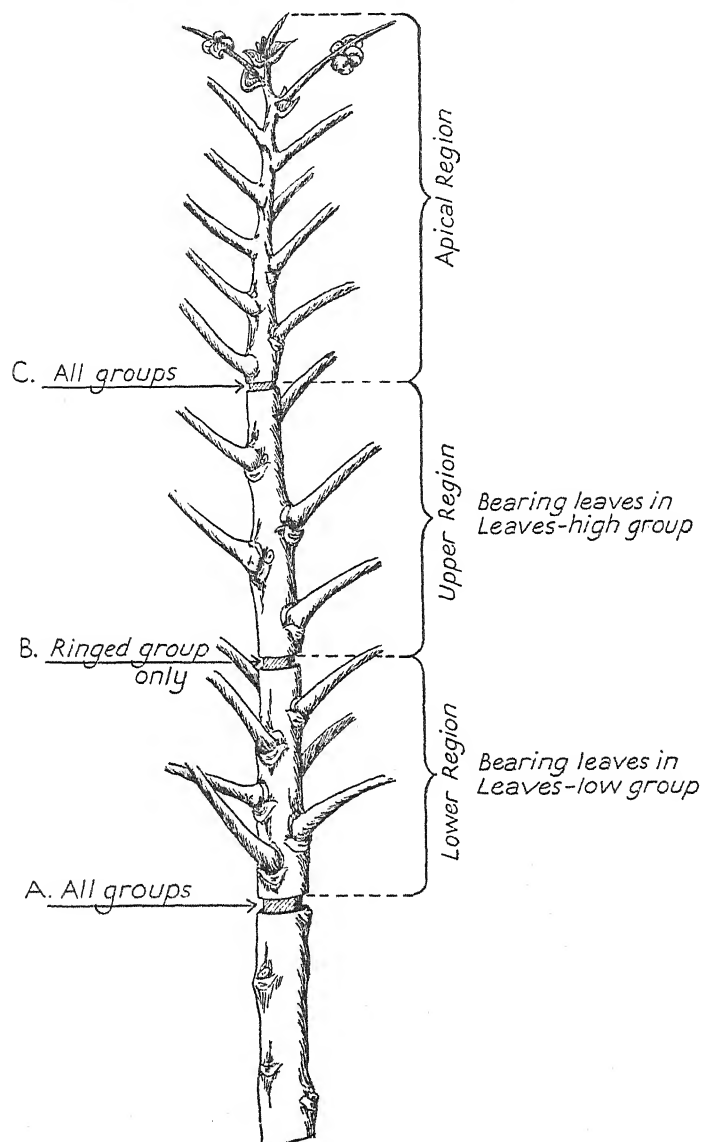


FIG. 1. Drawing illustrating the method of treating plants in Experiment 9.

Two samples, each of fifty-four plants, were collected from each group on the second day after ringing. Two similar samples were taken also from untreated plants immediately following the ringing. The time-table shows the sequence of operations.

Time-table.

Jan. 16, 1928.	6.30-7.30 a.m.	Ringings of plants at levels A and C.
" "	7.30-8.30 a.m.	Removal of leaves from Upper and Lower regions and ringing at level B in ringed group.
" "	9.0 a.m.	Initial collection of Normal plants (2 samples).
Jan. 18, 1928.		Collection of Leaves-high, Leaves-low, and Ringed groups.
" "	9.20 a.m.	Collection of 1st samples.
" "	1.22 p.m.	Collection of 2nd samples.

In sampling, the stems were cut in the field at levels A, B, C (Fig. 1), and trimmed in the laboratory at the stipular mark on the nodes next to the cut ends.

Results. The first observations were not made until more than forty-eight hours after ringing (the mean time was fifty-two hours). During that time an appreciable amount of growth was made in the groups bearing leaves. Both the dry weights and the residual dry weights for these groups were, in consequence, significantly greater than those for the Ringed group. The results are, accordingly, expressed on the sample basis, the amounts determined per sample being divided by the number of plants in the sample. A summary of the results is given in Table V (p. 141).

Since there was no significant difference between the estimates, for Upper and Lower regions, of the relative variances due to sampling, the estimates for the two regions have been pooled in order to have a general estimate based on all the observations. This estimate is given on the right of the table in the form of the standard deviation, expressed as a percentage of the mean for any pair of duplicate samples. The significance of the changes observed may be judged from Table VI, in which the difference between any two groups is expressed as a per cent. of the mean for the two groups (p. 143).

It will be noted (Table V) that the increase in *labile carbohydrates* (cf. p. 131) during the experiment is less than the increase in *total carbohydrate* (Dry weight -5.7N-ash), owing, presumably, to the transformation of labile carbohydrate to structural or non-hydrolysable material. Consequently the estimate of carbohydrate transport must be based on the figures for total carbohydrate.

To consider first the two sets of differences (columns *a*), Leaves-high minus Ringed in the Lower region, and Leaves-low minus Ringed in the Upper region, i.e. the differences indicating downward or upward movement via the bark, both the downward and the upward movement are as clearly established for phosphorus as for nitrogen and carbohydrate. The differences in total ash content point in the same direction. The differences do not reach the same level of significance as for phosphorus, but, taking into account the fact that the observed *excess* over the Ringed group would have occurred by chance in less than 5 per cent. of cases, it seems highly

TABLE IV.

Weights per 18 cm. Section of Stem in Low-ring and High-ring groups.

	(a)				(b)			
	Residual Dry Weight Basis.		Sample Basis.		Difference.		Significant Difference.	
	High-ring.	Low-ring.	Difference Low minus High.	P = 0.05.	Low minus High.	P = 0.05.	Low minus High.	P = 0.10.
Labile carbohydrates gm.	6.097	7.625	+ 1.528	0.162	+ 1.670 ¹	0.240		
Nitrogen mg.	387.9	430.2	+ 42.3	16.9	+ 49.4	13.5		
Ash "	1887.2	1899.4	+ 12.2	98.1	+ 49.0	106.2		85.7
Phosphorus mg.	133.9	145.6	+ 11.7	8.5	+ 14.7	9.8		
Potassium "	401.4	440.7	+ 39.3	23.9	+ 47.9	28.5		
Calcium "	197.5	192.7	- 4.8	7.42	- 2.02	11.15		9.06

TABLE V.

Weights per Plant in Upper and Lower Regions of Stem (Bark + Wood).

	Standard deviation as per cent. of the mean.				Standard deviation as per cent. of the mean.			
	Leaves-low.		Leaves-high.		Leaves-low.		Leaves-high.	
	Initial.	Ringed.	Initial.	Ringed.	Initial.	Ringed.	Initial.	Ringed.
Labile carbohydrates gm.	2.511	2.228	2.898	5.030	4.944	6.126	1.15	
Total carbohydrate "	8.463	8.474	9.272	18.578	18.560	20.176	1.52	
Nitrogen mg.	84.91	89.80	107.43	174.7	187.5	220.2	1.93	
Ash "	390.5	411.9	437.6	853.0	901.1	962.3	2.08	
Phosphorus mg.	43.19	45.48	49.80	87.78	92.78	106.67	1.92	
Potassium "	64.63	—	81.06	114.06	118.35	136.84	6.42	
Calcium "	50.65	51.09	50.85	115.15	120.47	126.60	2.32	

¹ Calculating the carbohydrate response from the figures for total carbohydrate (dry weight—5.7%N-ash) we obtain +1.852.

probable that movement, both downward and upward, of ash materials has occurred in the phloem. Data for the potassium content of the Upper region of the Ringed group are lacking, so that it is impossible to test upward movement of this element. Downward movement is apparently considerable (in percentage change potassium lies between phosphorus and nitrogen), but owing to the high sampling error the result is doubtful. Calcium shows only an insignificant change in the Upper region, and, while there is some suggestion of downward movement into the Lower region, this is not even partially significant.

To sum up, there is evidence for both upward and downward movement via the bark of phosphorus and ash. Downward movement of potassium in this experiment is not definitely established. It is very doubtful whether calcium has moved either upward or downward, but it does seem certain that this apparent immobility of calcium is not due to a cessation of calcium uptake by the roots, for, in the Lower region, the Leaves-high group shows a significant increase over the Initial group.

The series of differences between the Ringed and the Initial groups (Table VI, columns *b*) should be measures of uptake direct from the transpiration current in the wood. We can in this way obtain further evidence as to the channel of upward transport from the roots. We noted in the earlier paper (20) that both stem regions in the Ringed group showed an increase in total nitrogen over the Initial group, and suggested that this was due to uptake from the transpiration current. It will be seen (Table VI) that there is also an increase in ash, phosphorus, potassium, and calcium. None of these differences between Ringed and Initial groups, except that for nitrogen, reaches the higher level of significance ($P = 0.05$), but the increase in ash is just on the 10 per cent. level in both regions of stem, while the increase in phosphorus is above this level. If there were no real difference between Ringed and Initial groups the observed difference in ash and phosphorus content in *favour of the Ringed group* would have occurred by chance in only 5 per cent. of cases or less. It appears very probable, therefore, that there has been in the ringed plants direct uptake of phosphorus and other ash constituents by the stem tissues from the ascending transpiration current. Whether this direct uptake would occur to the same extent in plants in which the normal supply of solutes was being delivered by the leaves to the bark is, of course, doubtful. In our ringed plants this supply from the leaves via the bark had ceased. On this view the increases in nitrogen, phosphorus, and potassium shown by the Leaves-high and Leaves-low groups over the Initial group (Table VI, columns *c*) consist mainly of material which first travelled to the leaves in the transpiration current, and was then re-exported either upwards or downwards via the bark. The increase in calcium on the other hand, would be due to direct uptake from the transpiration

TABLE VI.
Relative Differences Between Groups.

	Upper Region.			Lower Region.			
	(a)	(b)	(c)	(a)	(b)	(c)	
	Leaves-low minus Ringed.	Ringed minus Initial.	Leaves-low minus Initial.	Leaves-high minus Ringed.	Ringed minus Initial.	Leaves-high minus Initial.	Significant Differences. P = 0.05, P = 0.10.
Total Carbohydrate	+ 8.99	+ 0.13	+ 9.01	+ 8.34	- 0.10	+ 8.30	4.93
Nitrogen	+ 17.88	+ 5.60	+ 23.42	+ 16.02	+ 7.10	+ 23.04	5.08
Ash	+ 6.06	+ 5.32	+ 11.40	+ 6.56	+ 5.48	+ 12.04	5.48
Phosphorus	+ 9.06	+ 5.17	+ 14.22	+ 13.93	+ 5.54	+ 19.32	5.05
Potassium	—	—	+ 22.55	+ 14.49	+ 3.69	+ 18.15	17.63
Calcium	- 0.47	+ 0.87	+ 0.39	+ 4.96	+ 4.52	+ 9.47	7.57

TABLE VII.
Distribution of the Response to Ringing as between Bark and Wood.

	(a) Distribution of amounts present.			(b) Distribution of response to ringing.		
	Expt. 2.	Expt. 3.	Expt. 9.	Expt. 2.	Expt. 3.	Expt. 9.
Mean amount of material present in the bark, expressed as a percentage of the mean amount for the stem (bark + wood).						
Total Sugars	75·6	66·7	68·3	70·9	65·0	93·6
Polysaccharides	35·3	25·3	24·9	72·3	58·8	60·4
Labile carbohydrates	49·0	34·5	38·4	71·2	62·5	67·6
Total carbohydrate	—	—	26·8	—	—	57·1
Nitrogen	44·2	49·6	47·4	58·5	34·0	46·2
Ash	46·7	47·9	51·2	36·4	—	37·3
Phosphorus	28·4	25·3	29·4	48·5	36·2	36·6
Potassium	36·5	37·5	33·5	21·2	20·4	39·4
Calcium	70·2	67·6	73·7	—	—	36·0
						(Up)
						Expt. 9.
						86·8
						45·5
						54·6
						56·1
						50·2
						35·6
						15·2
						—
						—

¹ Table VII *a*. Values for Experiments 2 and 9 are based on means for Upper and Lower regions of the Normal group: values for Experiment 3 on means for the High-ring and Low-ring groups. Table VII *b*. Values for Experiments 2 and 3 are calculated on the same basis as in Tables II *a* and IV *a*; values for Experiment 9 (down) are based on the set of differences, Leaves-high minus Ringed, and for Experiment 9 (up) on the set of differences, Leaves-low minus Ringed of Table V.

current. It will be seen that these increases are fully significant in every case except for calcium in the Upper region of stem and for potassium in the Lower region of stem. Upward movement of nitrogen, ash, phosphorus, potassium, and calcium via the wood can hardly be doubted.

SECTION IV. DISCUSSION OF THE RESPONSE TO RINGING.

The results of the three experiments involving ringing may be summed up as follows. There is no evidence of upward movement via the bark from the roots towards the foliage region, but there is definite evidence of upward movement of nitrogen, ash, phosphorus, potassium, and calcium via the wood. When a ring is made between the foliage region and the roots, nitrogen, ash, phosphorus, and potassium accumulate in the stem above the ring and diminish in the stem below. We take this to indicate downward movement, via the bark, of material which had travelled upwards to the leaves in the transpiration current. This type of response is clearly established for nitrogen and for phosphorus in all three experiments; for ash and for potassium in two experiments, the increases shown for each in the third case being within the limits of sampling error. For nitrogen and for phosphorus, and very probably also for total ash, this normal downward movement in the bark can be reversed by reversing the relative positions of the leafy and leafless regions of stem.

Some additional features of the response shown to ringing will now be considered, using the data from the three experiments.

(a) Response in bark and wood.

So far, in discussing the accumulation above and decrease below a ring, we have dealt only with the changes in the stem as a whole. The distribution of this response as between bark and wood, for the three experiments, is shown in Table VII, where it may be compared with the distribution, between bark and wood, of the total amount of material present in the region of stem employed. The total amount of material present in the stem is taken as 100, and the per cent. present in the bark is given in Table VII *a*: similarly the total amount of material that has moved through the bark, i.e. accumulated in the stem-tissues above or decreased in the stem tissues below a ring (Tables II *a*, IV *a*, and V), is taken as 100, and the accumulation or decrease in the bark alone is given as a percentage of the total for the stem (bark + wood). The percentages for the wood are in each case complements of those for the bark and need not be given.

We drew attention in a previous paper (18) to a difference in behaviour between carbohydrate and nitrogen in the response to ringing. In that series of experiments the response of carbohydrate was much greater in the

bark, while the response of nitrogen was greater in the wood. In the present series (Table VII *b*) there is a similar though not such a striking divergence. The carbohydrate response is very much greater in the bark, while the response of nitrogen is, on the whole, less in the bark than in the wood. In this respect nitrogen occupies a position intermediate between carbohydrate and the ash constituents, for the latter all show a much greater response in the wood. If we are right in interpreting the response to ringing as an accumulation of material normally moving downward from the foliage regions via the bark, the wood may form an important temporary storage region for these materials in their downward movement. Also, the stronger tendency of ash constituents and of nitrogen to move laterally for this temporary storage should mean a stronger damping of fluctuations in concentration of these materials in the conducting tissues of the bark caused by fluctuations in supply from the leaves or in demand by growing organs.

It is interesting to note (Table VII *a*) that the ash constituent for which we have the best evidence of downward transport in the bark, i.e. phosphorus, is mainly present in the wood, while calcium, for which we have no such evidence of bark transport, is mainly present in the bark. Potassium resembles phosphorus, though the disproportion between the content in bark and in wood is not so great, while total ash is intermediate between potassium and calcium.

(b) The Ratios of Materials in Downward Movement.

We pointed out in an earlier paper (18) that, as the whole of the carbohydrate required for new growth of the lower part of the plant is coming down from the leaves, comparison of the ratio of nitrogen to carbohydrate in downward movement via the bark with the ratio of nitrogen to carbohydrate present in the lower part of the plant, should enable us to say whether the downward movement of nitrogen in the bark was adequate to maintain normal growth, or whether some nitrogen must normally be taken up direct from the transpiration current and synthesized locally. We found in that work that nitrogen comprised roughly 2 per cent. of the dry weight of the Lower region of stem, whereas the weight of nitrogen moving downwards was on the average 2.75 per cent. of the weight of carbohydrate moving downwards. These figures suggested that the whole of the nitrogen required for stem growth might be coming down as combined nitrogen from the leaves.

Analysis, from this point of view, of the values obtained in the present series of experiments gives the results shown in Table VIII.

The values given in Table VIII *a* for Experiments 2 and 9 are means for the Upper and Lower regions of stem. The two regions differed very little. For Experiment 3 the values are the means for the one region of

stem (a lower region) in the High-ring and Low-ring groups. The values for carbohydrate in Table VIII *a* are in all cases for total carbohydrate, calculated as (Dry weight — 5.7N-ash).

TABLE VIII.

Comparison of relative amounts of materials present in normal stem with the relative amounts in movement along the bark.

	(a) Relative amounts present in stem.				(b) Relative amounts in movement along bark.					
	Expt. 2	Expt. 3	Expt. 9	Mean	Expt. 2	Expt. 3	(Down) (Up)		Mean	Mean (S)
							Expt. 9	Expt. 9		
Carbohydrate	100	100	100	100	100	100	100	100	100	100
Nitrogen . .	1.58	1.44	0.98	1.33	2.51	2.77	2.02	2.21	2.47	2.58
Ash	7.64	6.65	4.61	6.30	7.72	0.80	3.78	3.32	4.01	4.70
Phosphorus .	0.52	0.49	0.49	0.50	0.51	0.77	0.86	0.54	0.66	0.78
Potassium . .	1.73	1.48	0.69	1.30	1.41	2.57	1.14	—	1.71	1.74
Calcium . . .	0.75	0.69	0.61	0.68	0.02	0.31	0.38	0.03	0.04	0.21

The values in Table VIII *b* are calculated from those given in Table II *a*, IV *a*, and V. Hence, for Experiments 2 and 3, in which the residual dry weight basis was used, the carbohydrate in movement is calculated from the figure for labile carbohydrates. The mean values for the three experiments are given on the right of Tables VIII *a* and VIII *b*. In the latter, downward and upward movement in Experiment 9 are considered as one experiment and the mean values taken. For comparison we give also, as Mean (S) in Table VIII *b*, the mean values for Experiments 2, 3 and 9, using the sample basis throughout and calculating carbohydrate in each case as (Dry weight — 5.7N-ash).

Before examining the data in the table some comment may be made on the use, as estimates of the ratios required to maintain normal growth of the lower part of the *plant* of the ratios of materials found in the lower part of the *stem*. The ratios observed in the lower part of the stem will be an underestimate or an overestimate of the requirement according as the corresponding values for the root are higher or lower than those for the stem. For the cotton plant we have found, in subsequent work (24), that, from the tenth week onwards, the ratios for nitrogen and for phosphorus are lower in the main roots than in the stem, while the values for ash, potassium and calcium are very little greater in the root than in the stem. Further, in both stem and root all the ratios steadily diminish as the plants age,¹ so that the ratios of other materials to carbohydrate in the *growth increments* must usually be well below the corresponding ratios for the

¹ Compare, for instance, the values in Table VIII *a* for Experiments 2, 3, and 9, which represent progressively older plants.

materials already present. Again, we have taken no account of the additional carbohydrate required to make good the loss by respiration. On all grounds it would appear that the ratios given in Table VIII *a* are over-estimates of the ratios required in the normal growth increments.

It will be seen from Table VIII that the amount of nitrogen moving down from the leaves is consistently in great excess of the estimated requirement, while the amount of phosphorus is always either equal to or greater than the estimated requirement. Potassium is more variable, but this too is, on the average, well in excess of the estimated requirement. Ash is apparently in one case up to the requirement, but on the average falls somewhat below. Calcium is clearly quite inadequate, the mean figure being negative. Considering that the values in Table VIII *a* are, in all probability, over-estimates of the requirements, this result for nitrogen, phosphorus, and potassium is very striking, and, provided we are right in regarding the material accumulating above a ring as material in downward movement via the bark, has implications of some importance. The basis for this interpretation must, therefore, be re-examined.

We referred earlier to the possibility that some materials might accumulate above a ring, not because they were moving downwards from the leaves, but because other substances, e.g. carbohydrates, were moving downwards, and by their accumulation above the ring caused an increased uptake, directly from the transpiration current, of the material in question. It is true that the accumulation of any substance causing increased growth should increase the rate of uptake of other materials required for growth. But, as we have already suggested, where there is no tissue growth (i. e. change in the residual dry weight), and this is the case in Experiments 2 and 3, no hunger (change in gradient) should be generated for the solutes of the transpiration current.

In the second place, if the accumulation of these materials is a consequence of the accumulation of carbohydrate, we might reasonably expect their accumulation to be *relatively* less than that of carbohydrate. We find, however (Table VIII, cf. *a* and *b*) that the relative accumulation of carbohydrate falls below that of nitrogen, phosphorus, and potassium; i. e. these elements accumulate in greater proportions than those required for growth. The accumulation of total ash is relatively less than that of carbohydrate, but this may well be due to the fact that total ash includes some materials, such as calcium, which are not re-exported in appreciable quantities from the leaves, but are derived mainly by direct uptake from the transpiration current. Thirdly, if the materials other than carbohydrates accumulating above a ring are derived directly from the transpiration current, the supply of these materials to the leaves should be reduced in the ringed plants. Actually we find, Table III, an accumulation of all these materials in the leaves of the ringed plants, the excess being

statistically significant for nitrogen and for total ash. Since import of tracheal sap into these leaves has probably diminished somewhat, this accumulation can only be due to decreased export via the bark; and it seems reasonable to connect this with the increased concentration in the bark above the ring, which should tend to diminish the effective gradient of export from leaf to bark.

In the case of nitrogen we have still more conclusive evidence in favour of downward movement via the bark. In the Bark Flap Experiment (18) we showed that nitrogen can travel down from the foliage region and accumulate in long flaps of bark quite isolated from the wood. A similar experiment has not yet been carried out for the ash constituents, but, in view of the other evidence noted above, there seems little reason to doubt that the observed accumulation of certain ash constituents, including phosphorus and potassium, above a ring is, like the accumulation of nitrogen and of carbohydrates, due mainly to the downward movement of these materials from the foliage via the bark.

The implications of the apparent excess of nitrogen, phosphorus, and potassium, as compared with carbohydrate, in downward movement from the leaves must now be examined. A possible explanation is that the manufacture of carbohydrate in the ringed plants is soon hindered by the accumulation of sugar which occurs in the leaves above the ring. Nitrogen, phosphorus, and potassium and other ash constituents would continue to arrive via the wood at very nearly the normal rate, while the rate of synthesis of such as these as form organic compounds might, owing to the higher sugar concentration, actually increase. The data obtained in Experiment 2, when collections were made 5.5, 11, and 24 hours after ringing, are barely adequate to test this suggestion, but so far as they go they are against it; for the ratio of the nitrogen and the ash response to the carbohydrate response actually diminished in time, while the responses of phosphorus and of potassium showed no definite drift. Again, in more recent experiments, lasting for several days, we have found that the ratio of nitrogen to carbohydrate in the material accumulating above a ring steadily diminishes from the first day onwards.

It appears very probable, therefore, that in the downward movement of material from the leaves via the bark, nitrogen and phosphorus and possibly also potassium and some other ash constituents are in excess of what is required for growth of the lower part of the plant. What becomes of the excess? May it not be liberated into the transpiration current and so again ascend towards the foliage region?

(c) *Composition of the Tracheal Sap.*

In this connexion it will be interesting to consider what information we have as to the composition of the tracheal sap. Observations were made

on the tracheal sap in the main axis of plants of about the same age and from the same population as those used for the Reversal Experiment. The tracheal sap was obtained by the water-displacement method described previously (23), with the exception that dilute starch solution was used instead of eosin solution as the displacing liquid. The first few drops from the lower end of the stem were rejected, and the cut surface blotted dry with filter paper. Collection was then continued until small fractions taken from the hanging drops on the cut end showed a positive iodine test for starch. Usually 10-15 c.cs. were obtained before the starch appeared. Using starch solution coloured with eosin, the colour due to the eosin was clearly visible 2 or 3 drops after the first positive test for starch. Using either starch solution or eosin, admixture of the sap with the displacing solution would thus be very slight. The results are given in Table IX.

TABLE IX.

Concentrations per 100 grm. water in the Tracheal Sap.

Total solids	123.3 mg.
Total sugars	15.5 "
Total nitrogen	12.16 "
Ammonia N	2.37 "
Nitrate N	5.36 "
Organic N	4.43 "
Calcium	11.64 "
Phosphorus	3.86 "

The value obtained for sugars is rather low, previous work using eosin solution having given 43 mg. (23, p. 47). Additional values, obtained during the present experiments by the eosin solution method, for phosphorus and calcium were roughly of the same order as in the table, viz. 8.14 mg. for calcium and 3.75 mg. for phosphorus. These results for the cotton stem are similar in general level to those obtained by Anderssen (2) using tracheal sap obtained by the gas displacement method from branches of pear. Important features of the results are (1) the very low ratio of sugar to the mineral elements, as compared with the ratio in living cells or in downward movement through the bark, and (2) the high proportion of organic nitrogen. Anderssen reports that in his stems the whole of the nitrogen was organic, but it is not clear whether or not his organic nitrogen includes ammonia. He notes also that the concentration of phosphate in the tracheal sap of the pear increases markedly in the spring and suggests that much of it must have come from the surrounding tissues. Thus the composition of the tracheal sap, notably the relatively low concentration of sugars and the presence of organic nitrogen, supports the suggestion that, while the greater part of the sugar descending the stem is used up in growth, storage and respiration, the nitrogen, phosphorus, and possibly other

mineral elements moving down, are only in part used for growth and storage, leaving a residue which is liberated into the transpiration current.

(d) *The Role of the Transpiration Current in the Transport of Mineral Nutrients.*

The foregoing observations suggest the following general picture of the transport of mineral nutrients in cotton. Nutrients absorbed by the root are liberated into the tracheae of the root, where, so long as transpiration is active, they are maintained in relatively low concentration. They ascend the stem with the transpiration current, and may pass laterally into the living cells of the wood and into the bark tissues. Under normal conditions, however, a large proportion passes, along with the bulk of the water, to the leaves, where concentration inevitably takes place, probably accompanied in the case of nitrogen, phosphorus, and some other elements, by elaboration into organic compounds. From the leaf most of these materials may be re-exported via the phloem to other parts of the plant. In the case of materials moving downwards towards the roots, these may also move laterally into the other tissues of the bark and into the wood, where they are in part used for new growth or storage, but in part also leak into the tracheae and so ascend again with the transpiration current. Materials moving towards developing foliage or fruits, on the other hand, would be used entirely for growth or storage. For some elements, such as calcium, re-export from the leaf would seem, if it occurs at all, to be very slow, so that the supply of calcium to any tissue would be mainly by direct uptake from the xylem. Probably other elements occupy an intermediate position between nitrogen, phosphorus, and potassium on the one hand, and calcium on the other. If transpiration were completely prevented, upward movement of nitrogen, phosphorus, potassium, and probably some other elements, might take place via the phloem, though upward movement, via the xylem, of water utilized for growth might also contribute to the upward movement of materials.

If this picture of the cycle of transport should prove applicable to higher plants in general, it may be a means of reconciling the upward movement of soil nutrients in the transpiration current with the fact that a considerable increase in transpiration rate may have only a very small effect on uptake of salts by the roots. An adequate theory of uptake of nutrients by the root has not yet been developed, but it seems probable that rate of uptake does depend in part on the concentrations in the root cells and in the external solution respectively. The increased rate of uptake obtained by Brezeale (4) and by other workers (9) after a period of starvation may be instanced in this connexion.

Now although, as Curtis (8) points out, rapid upward removal of nutrients, by means of the transpiration stream, from the neighbourhood of

the absorbing tissues, should tend to *increase* the rate of uptake by the roots from the external solution, the return of these nutrients from the foliage region via the phloem should tend to *diminish* uptake. This damping effect, upon net uptake, of the return of nutrients towards the roots should be greatest in the case of those nutrients, such as, perhaps, potassium, that are re-exported from the foliage region practically unchanged, i. e. as free ions or as loosely combined ions. The damping effect might be smaller for nutrients, such as ammonium, nitrate, and phosphate ions, that are chemically transformed within the plant. In view of the known formation of ammonia within the plant by degradation of organic compounds, some effect of the incoming organic nitrogen on the net uptake of ammonium ions by the roots might be expected. Where inorganic nitrogen was being supplied as nitrate the damping effect should, presumably, be smaller. Organic phosphorus compounds might behave similarly to organic nitrogen compounds, but, since phosphorus very largely exists in the plant in the form (phosphate anion) in which it is absorbed, this element should, on the whole, be intermediate in behaviour between potassium and nitrogen. On the other hand, for elements such as calcium that are not re-exported from the foliage region via the phloem, there will be no increased return of materials to the root to compensate for increased rate of upward removal in the transpiration current.

To sum up, if the return of nutrients from the foliage via the phloem to the roots is a general phenomenon, we should expect, on increasing the transpiration rate, a more rapid circulation of materials and some increase in general level of concentration, but only a relatively small increase in net rate of uptake from the external solution, except in the case of elements that are not re-exported from the foliage region or that undergo irreversible chemical transformation in the plant.

If, however, as in Muenscher's (25) light-shade experiments, the increased transpiration is brought about in a manner that involves also an increased rate of carbon assimilation, the utilization of nutrients for tissue growth will be increased, and net uptake by the roots must increase. In Muenscher's experiments this effect completely overshadows the transpiration effect, and in fact it is not even clear whether transpiration rate has kept pace with the increasing size of the plant. The percentage increases of the light plants over the shade plants were: (i) Summer series: final ash free dry weight + 154; final ash content + 166; total transpiration + 108. (ii) Winter series: final ash free dry weight + 96.5; final ash content + 109; total transpiration + 72.5. In Muenscher's dry-humid series, on the other hand, only the transpiration effect is appreciable, the percentage increase of the dry over the humid plants being: Final ash free dry weight + 1.0; final ash content + 8.9; total transpiration + 106. Similar increases in salt uptake have been observed in dry-

humid experiments by Prát (27), the increases in uptake being 32, 6, 26, and 13 per cent. for increases in transpiration of 164, 175, 372, and 743 per cent.

If we are correct in thinking that the purely transpirational effect is restricted mainly to materials, such as calcium, that are not re-exported from the foliage region, the relatively small effect on *total* salt uptake is, in part, explained. Even for these materials, however, we should not expect an increase in uptake *proportional* to the increase in transpiration rate. Increasing the transpiration rate can only increase rate of upward removal of materials by steepening the gradient from the root-cells into the tracheal sap. As the transpiration rate increases this gradient approaches a limiting value, since the concentration in the tracheal sap cannot fall below zero. We should, therefore, have a law of diminishing returns connecting transpiration rate and rate of upward removal of salts, direct proportionality being approached only at the origin of the curve where transpiration rate was zero.

Whether this relation, or the return of certain nutrients towards the roots, plays the more important part in determining the relatively small effect of transpiration rate upon total salt uptake cannot, unfortunately, be tested on the data available, which are for total salt uptake only. Reference may, however, be made to some indirect evidence afforded by the results of Domontowitsch and Groschenkow (9), who measured the yield of plants that were supplied with certain nutrients for only a few hours every other day. During this period some plants were kept in the light, others in the dark. The yields obtained were greater for the light series, but the percentage increase in yield varied with the nature of the nutrient that was being supplied for the short period only. The increase was greatest with calcium and least with potassium, the full order being Ca, Mg, NO_3 , S, NH_4 , P, K. Assuming, as the authors do, that this effect on yield was due to increased uptake of the differentially supplied nutrient, the order found is very suggestive of a transpiration effect of the type we are discussing. The order is roughly the inverse order of the capability of the nutrients for re-export from the foliage (cf. p. 151). It must be said that the authors themselves, assuming that any transpiration effect should be the same for all nutrients, prefer to regard the results as an effect of light intensity. In view of the present discussion it would appear that an effect of transpiration cannot be so simply excluded.

In conclusion, it is clear that the known facts as to the relation between transpiration rate and net uptake of nutrients do not form any serious objection to the upward movement of these nutrients in the transpiration current.

(e) *Concentrations of Mineral Elements in Leaf, Bark, and Wood, and Vertical Gradients in the Bark.*

In view of the conclusion that some ash constituents, including phosphorus and potassium, resemble nitrogen, in that they are re-exported from

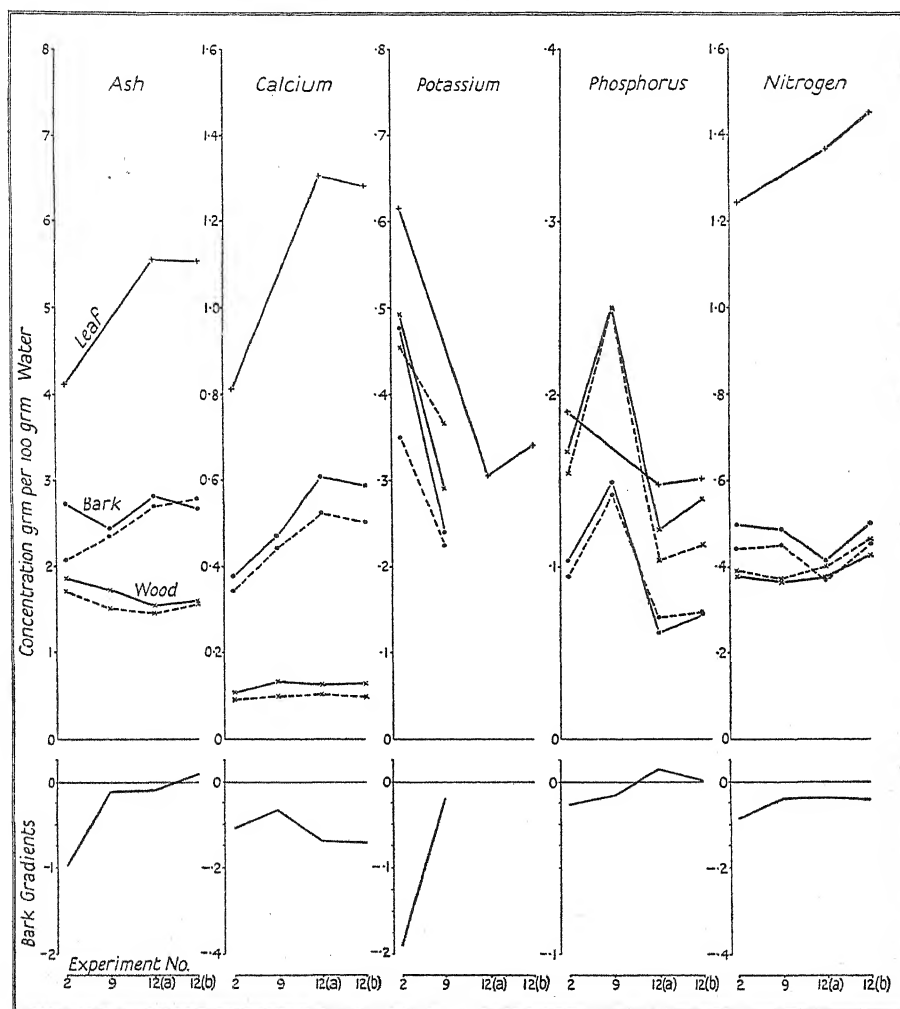


FIG. 2. Concentrations, grammes per 100 grammes water, in leaf, bark, and wood.

the leaves and travel downwards in the bark, whereas others, such as calcium, show no evidence of re-export and movement via the bark, it is of some interest to examine the tissue distribution of concentrations for these elements. The data available are, of course, only for total amounts present per 100 grammes water, and include structural and storage material as well as

mobile material. In Fig. 2 we give the mean values for the normal or unringed plants in Experiments 2, 9, 12a, and 12b. Experiment 12 was carried out on plants which were allowed to develop flowers and bolls, and will be discussed in the next section. It is included here in order to complete the data on concentrations and vertical gradients. 12a is the Bolls-on series and 12b the Bolls-off series (see p. 157).

Leaf concentrations are shown by the vertical crosses. No leaf data are available for Experiment 9. In the case of bark concentrations (solid circles) and wood concentrations (crosses), the concentrations in the Upper region are indicated by the broken line and those in the Lower region by a continuous line. At the foot of the graph are shown the vertical gradients in the bark (Upper minus Lower) calculated for a 30 cm. interval between the midpoints of the two regions. Negative gradients are measured downwards from the zero line. The vertical scales are the same for the gradient graphs as for the concentration graphs, but for the different materials the relative scales are: ash $\times 1$, nitrogen and calcium $\times 5$, potassium $\times 10$, phosphorus $\times 20$.

To consider first the relative concentrations in wood, bark, and leaf, calcium shows the greatest divergence, the mean concentrations in wood, bark, and leaf being roughly in the ratio 1:4.3:10.3. Ash is similar, though the divergence is not nearly so extreme, the ratios being 1:1.6:3.2. For phosphorus and potassium, on the other hand, the concentrations are more nearly equal in the three tissues, and the wood has a higher concentration than the bark. The average ratios are: for potassium 1:0.8:1.3, and for phosphorus 1:0.6:1.2. This contrast between calcium and total ash on the one hand and phosphorus and potassium on the other would seem to be in accord with our conclusion that phosphorus and potassium are re-exported from the leaf, whereas calcium does not appear to move down from the leaves. The relative accumulation of total ash as well as calcium in regions where transpiration is most active suggests that some other ash materials may resemble calcium in being remobilized very slowly if at all from the tissues which they reach via the transpiration current.

Turning to the vertical concentration gradients in the bark it will be seen that these are, in general, negative. In the case of nitrogen we have given reasons (20) for thinking that the observed gradient in the bark is the resultant of two components: (1) a dynamic gradient of mobile or translocatory material in the conducting cells; (2) a static gradient of immobile or storage material, mainly situated in the rays and cortex of the bark, but perhaps partly also in the conducting cells themselves. The dynamic gradient is, of course, positive when downward movement is in progress, and becomes negative only when, as in the Reversal experiment, movement is reversed. The storage gradient in the case of nitrogen is persistently

negative, the concentration of stored material being greater in the Lower regions of bark, which are also, of course, the older¹ regions. The storage gradient outweighs the dynamic gradient so that the net gradients observed are usually negative. A similar state of affairs may possibly hold for phosphorus and for potassium. In this connexion it is interesting to note that there is some suggestion of a connexion between the gradient and the mean level of concentration maintained. For the potassium the negative gradient drops almost to zero as the concentration drops from Experiment 2 to Experiment 9, while for phosphorus the gradient becomes slightly positive as the concentration falls from Experiments 2 and 9 to Experiment 12. It seems possible that, so long as the supply of an element is relatively abundant, the cells of the bark continue to store the surplus that is not required for immediate growth. A negative storage gradient would then arise in the bark, partly owing to the longer time during which storage had progressed in the tissues at the base of the stem and partly owing to the more rapid rate of uptake by the roots during earlier phases of development. If, however, supply were restricted, there might be no appreciable storage, and the vertical gradient in the bark would then be positive, representing the positive dynamic gradient with its transport head in the foliage region. If this type of behaviour could be established, it might be possible to determine whether the supply of an element was deficient or in excess by examining the vertical gradients in the bark.

In the case of calcium it is uncertain whether the material that accumulates in the leaf or bark can be remobilized: we can hardly therefore use the term *storage*, which implies the possibility of re-utilization. On account of their greater age the tissues at the base of the stem should have accumulated more calcium and a negative gradient should be present. But, in contrast to phosphorus and potassium, a restricted supply should not cause a disappearance or a reversal of the net gradient. It will be noted that the calcium gradient is, in fact, consistently negative throughout, and shows little change though the average concentration varies considerably.

In one experiment (Experiment 9) we have data for the vertical gradients in inner and outer halves of the bark of normal plants (Table X).

For calcium the vertical gradients are similar in the two halves, but for phosphorus and for potassium the inner half shows a positive gradient which is masked, in the bark as a whole, by the steep negative gradient in the outer half. This suggests that storage is occurring in rays and cortex, producing a negative static gradient in these tissues, while the gradient in

¹ We owe to Dr. F. F. Blackman the suggestion that the storage gradients, which we postulated in our presentation of the relation between longitudinal transport of nitrogen and the net gradients in the bark, might be associated with the greater age of the basal tissues. Following out this suggestion we have made some observations on the ontogenetic drift of vertical gradients in the main axis: the results of these observations will be presented in a subsequent paper.

the sieve-tube region represents the positive dynamic gradient of translocatory materials. Further observations are of course required to test this suggestion.

TABLE X.

Concentrations and Gradients in Inner and Outer Halves of the Bark.

		Concentrations.				Gradients.		
		Inner half.		Outer half.		Inner	Outer	Whole
		Upper.	Lower.	Upper.	Lower.	Half.	Half.	Bark.
Ash	gram.	2.494	2.503	2.188	2.374	- 0.009	- 0.186	- 0.098
Phosphorus	mg.	164.9	163.0	119.2	135.5	+ 1.9	- 16.3	- 7.3
Potassium	"	259.5	232.8	186.2	246.2	+ 26.7	- 60.0	- 16.7
Calcium	"	453.9	488.8	425.0	449.4	- 34.9	- 24.4	- 29.7

The questions of storage and of dynamic gradients in the phloem cannot be taken much further until we can deal with sap concentrations as well as total concentrations, and determine how the material is distributed as between insoluble, adsorbed, and freely mobile fractions. In the present experiment the dried material available was not sufficient to permit even of a simple water soluble determination for each sample. The material remaining after the total estimations was, however, bulked so as to have a determination of the water soluble fractions for the three tissues—leaf, bark, and wood. The results are given in Table XI.

TABLE XI.

Water-soluble Phosphorus, Potassium, Calcium in Leaf, Bark, and Wood.

(Water soluble fraction expressed as a per cent. of the total.)

	Leaf.	Bark.	Wood.
Phosphorus	57.1	72.0	89.2
Potassium	99.4	94.5	86.8
Calcium	73.8	15.0	25.2

The higher proportion of soluble calcium in the leaf, which is in accord with the observation of Reed and Haas (29) on citrus, is somewhat surprising in view of the apparent inability of the leaf to export calcium to the bark. Similarly the very high proportion of water-soluble potassium raises the question as to how this element is stored. The cations leached out by water may of course be, in part, adsorbed cations released by the hydrolytic action of the water;¹ in any case the destruction of cell structure in killing and drying may set free substances which in the living cell would not be free to move.

SECTION V. MOVEMENT TO THE BOLL.

In the scheme of work on nitrogen transport in cotton a study of certain aspects of boll-growth was included. The results obtained have

¹ Cf. the effect of leaching a soil.

been discussed, in the light of the other observations on nitrogen transport, in an earlier paper (22). Ash determinations have now been made on the material, and the distribution of ash, phosphorus, potassium, and calcium will be discussed in the present section. Two experiments were carried out. In the first we studied the effect of removing the growing bolls on the concentration of nutrient materials in the leaves and in the stem tissues; in the second we studied the uptake of materials during the first seven days' growth of fertilized and unfertilized bolls.

(a) *The Effect of Removal of the Bolls on Concentrations of Materials in Leaf, Bark, and Wood (Experiment 12).*

Procedure. The plants were fourteen and a half weeks old, and were in the fifth week of flowering. The mean height was 95.7 cm., and the mean number of bolls per plant 15.3. Samples were drawn for two groups of plants. From the plants of one group, Bolls-off group, all flower-buds, flowers and bolls were removed on February 23, 1928. The other, Bolls-on group, was not touched. Five days later, three samples, each of twenty-five plants, were collected from each group. The material taken for analysis was: (1) Upper region: the stem between the seventh and twelfth nodes from the apex and the basal leaf on each of the three middle fruiting-branches of this region; (2) Lower region: the stem between the first and seventh nodes, counting upwards from the lowest fruiting-branch, and the basal leaf on each of the three middle fruiting-branches of this region. The mean distance between midpoints of Upper and Lower regions was 35.9 cm.

In addition to this set of samples for the analysis of *selected regions* of the plant, a set was taken for analyses of the *whole plant* above the ground. The latter was subdivided for analysis into upper and lower halves of the main axis, fruiting-branches, leaves, petioles, flower-buds, and bolls. Two samples were taken on February 23, at the start of the experiment, and two more from each group five days later. Ash analyses were not made on this material but only total nitrogen determinations. As, however, these determinations provide evidence as to the net uptake of a soil nutrient by the tops of the plants during the experiment, they have a bearing on the results obtained for the selected regions analysed in the main series.

Results. The results for the *selected regions* for the plant are shown in Table XII. The mean excess or deficit per sample in the Bolls-off group is expressed as a per cent. of the mean amount found in the Bolls-on group.

A word should be said concerning the significant differences placed in brackets in the table. As there was not sufficient material for determination of the ash constituents in the individual bark samples, the determinations had to be carried out on the bulked samples; we have, therefore, no

TABLE XII.

Percentage increase of Bolls-off over Bolls-on group in weight per sample.

	Fresh weight.	Total carbohydrates.	Labile carbohydrates.	Total nitrogen.	Ash.	Phosphorus.	Calcium.	Water.
<i>Leaf.</i>								
Upper region	+7.28	+9.45	+22.6	+10.38	+8.25	+7.19	+8.41	+6.49
Lower region	+3.55	+3.70	+13.3	+12.63	+0.36	+7.13	-1.93	+3.10
Significant Differences:								
P = 0.05	3.36	5.51	6.55	7.69	4.94	7.56	10.20	2.96
P = 0.10	.	4.37	.	.	3.79	6.00	8.10	.
<i>Bark.</i>								
Upper region	+15.30	+18.7	+32.5	+39.3	+17.36	+18.09	9.47	+13.9
Lower region	+4.16	+7.9	+14.1	+25.0	-2.34	+23.82	0.14	+2.93
Significant Differences:								
P = 0.05	7.19	7.49	15.5	10.77	(17.36)	(9.20)	(10.69)	7.25
P = 0.10	5.79	.	12.5	.	(14.01)	(7.31)	(8.49)	5.86
<i>Wood.</i>								
Upper region	+10.7	+6.15	+12.4	+28.8	+21.15	+22.30	+3.43	+20.5
Lower region	+4.59	+0.57	+8.6	+20.4	+10.59	+22.85	+10.10	+6.25
Significant Differences:								
P = 0.05	7.97	7.31	12.6	9.49	17.36	9.20	10.69	8.25
P = 0.10	6.44	5.90	10.0	.	14.01	7.31	8.49	6.65
<i>Stem (Bark + Wood).</i>								
Upper region	+12.6	+10.20	+21.0	+33.0	+18.95	+20.85	8.21	.
Lower region	+4.42	+2.53	+10.8	+22.5	+3.05	+23.15	+2.02	.
Significant Differences:								
P = 0.05	7.55	7.56	13.4	9.61	(17.36)	(9.20)	(10.69)	.
P = 0.10	6.10	6.10	10.7	.	(14.01)	(7.31)	(8.49)	.

direct estimate of sampling variation for the bark. Since, however, the percentage variation, due to sampling, for the wood is, in our experience with cotton, not appreciably different from that for the bark, we have felt justified in using for the bark and for the stem (bark + wood) the significant differences calculated for the wood. Potassium figures were obtained for the leaves only, and the response of potassium will not, therefore, be considered.

The results obtained for dry weight and for total nitrogen for the series in which the whole plant above ground was sampled are given in Table XIII. The figure for 'Bolls' includes flower-buds and flowers, the figure for Bolls-off group representing flower buds that appeared during the experiment.

It is clear from Table XIII that nitrogen continued to be taken up from the roots in both groups of plants but that, in the Bolls-on group, import into the lower half of the stem was insufficient to make good the drain by the bolls. Consequently, in discussing the differences shown in Table XII, between Bolls-on and Bolls-off groups, although the greater part of the response of nitrogen and probably also of phosphorus and ash must be ascribed to material taken up and elaborated during the experiment, part must also be ascribed to the remobilization of material present as storage material in the stem when the experiment began.

TABLE XIII.

Dry Weight and Total Nitrogen in Aerial parts of plants.

	Dry Weight.			Total Nitrogen.		
	Initial.	Bolls-on.	Bolls-off.	Initial.	Bolls-on.	Bolls-off.
Total . . .	137.0	158.4	108.35	4.046	4.385	2.947
'Bolls' . . .	39.29	53.30	0.85	1.315	1.596	0.035
Total minus Bolls	97.71	105.10	107.5	2.731	2.789	2.912
Stem (excluding Apical Region):						
Upper half . .	7.00	9.34	9.04	0.0692	0.0786	0.0987
Lower half . .	16.95	16.06	17.14	0.1497	0.1352	0.1685

As noted in the earlier paper (22), removal of the bolls is in some respects like ringing, since in both cases movement of material via the phloem is interrupted, in one case by removal of a 'sink', the bolls, in the other by isolation of a sink, i.e. the lower part of the plant, from the source, i.e. the foliage region. In both cases we expect, on the gradient view of transport, an accumulation of materials in the supplying (leaf) and conducting (stem) tissues. Table XII shows that this expectation is realized. In the leaves of the Upper region the response is very nearly the same for total carbohydrate, nitrogen, ash, phosphorus, and calcium, but labile carbohydrates show a relatively greater increase. In the Lower region the response of nitrogen and phosphorus is greater than that of total

carbohydrate, but about the same order as that of labile carbohydrates. The responses of ash and of calcium are negligible.

In the stem tissues, as in the leaf, the responses are greater in the Upper region. The nitrogen response is the greatest and is well marked in both regions of bark and wood. The phosphorus response, though smaller than the nitrogen response, is again significant in every case and, except in the Upper region of the bark, exceeds the response of labile carbohydrates. The ash response is significant in the Upper region of the stem but not in the Lower region. Calcium shows a partially significant response in the Upper region of the bark and the Lower region of the wood. In the stem as a whole the calcium response is not even partially significant.

From the point of view of the effect of the bolls on the growth of the rest of the plant the results indicate that we must take into account the demand of the boll for phosphorus as well as its demand for nitrogen and for carbohydrates. This confirms a suggestion made in the earlier paper (22).

The consistent responses of carbohydrates, nitrogen, and phosphorus in leaves and stem in both regions, and the consistent response of ash also in the Upper region, would seem to be in harmony with the view that these materials are elaborated in the leaf and travel along concentration gradients in the phloem to the boll. That the observed responses represent the accumulation of materials in course of transport is strongly supported by the fact that the observed increases are usually much greater than the accompanying increases in fresh weight, which we may take as a rough measure of growth. Again, in leaf and bark the increase in water-content is less than the increase in fresh weight, so that the observed increases of carbohydrates, nitrogen, phosphorus, and ash represent increases in sap concentration in these tissues.

It will be noted that, in the Lower region of the plant, the responses of nitrogen and of phosphorus are much greater than the response of carbohydrate. It seems probable that the response in the Lower region is partly due to the accumulation in the Bolls-off group of nitrogen and phosphorus which would otherwise have migrated to the boll, and partly also to the withdrawal, in the Bolls-on group, of nitrogen and phosphorus stored in this region of the plant. This suggestion is confirmed, as far as nitrogen is concerned, by the data in Table XIII. The upper half of the stem increases in dry weight and total nitrogen in both groups. The lower half of the stem shows a small net loss in dry weight in the Bolls-on group but a much greater percentage loss in nitrogen. About 40 per cent. of the nitrogen difference between Bolls-off and Bolls-on groups for this part of the stem is due to this withdrawal of nitrogen in the Bolls-on group.

If this is the correct explanation of the greater response of nitrogen and phosphorus, as compared with carbohydrate, in the Lower region the results would appear to confirm our suggestion that the negative vertical

gradients of nitrogen and of phosphorus in the bark may be due to greater storage in the Lower region. The apparent response of calcium, which is partially significant in leaf and bark of the Upper region, and in the wood of the Lower region, is somewhat unexpected, in view of the fact that its transport seems to be restricted mainly to movement in the transpiration current. It is possible, of course, that the greater supply of sugars to the roots of the Bolls-off plants has caused an increased uptake of all mineral nutrients, including calcium. In fact all the increases observed may in part be due to this cause. It will be noticed, however, that the calcium response is usually much smaller than that of phosphorus or even total ash, and that it is in most cases much less than the increase in fresh weight. On the whole our conclusion, from the ringing experiments, that comparatively little movement of calcium takes place in the bark, is confirmed by the present experiment.

(b) *Uptake of Materials by the Young Boll (Experiment 15).*

In this experiment (cf. 22), the growth of the ovules and carpel walls of the boll was followed for seven days from flowering in fertilized and unfertilized bolls. The object was to see whether a study of sap concentrations in the growing organs would throw any light on the conditions governing uptake of nitrogen and carbohydrate. For the ash constituents we have, of course, only determinations of the total amounts present, and the nature of the gradients from bark to boll cannot be examined. There are, however, two points of some interest in connexion with the general problem of transport of ash constituents. These concern (1) the varying effect of fertilization on the uptake of different materials and (2) the uptake of materials by the normal ovule in relation to its uptake of water.

Procedure. A large number of flowers were tagged with coloured wools on the evening before they opened. In approximately half the total number tagged on each day fertilization was prevented by removal of the stigmas at dawn, before the corolla opened. The normal and unfertilized bolls were subsequently collected in samples of about four hundred bolls for each. Four such samples were taken during each day. The bolls were trimmed at the base, and then subdivided into *ovules* and the remaining tissues, which we shall refer to as *carpels*. There were two series of collections, Series A with bolls 1, 3, 5 and 7 days old, and Series B with bolls 0, 2, 4, and 6 days old. The time-table shows the arrangement of collections.

Results. The uptake of materials by the ovules and the carpels of normal and unfertilized bolls is shown in Fig. 3. Series A and B have been combined. The values plotted are the logarithms to the base 10 of the weights per 100 bolls. This method of plotting enables one to compare relative changes very simply, since the vertical scales used are the same for all materials. The materials are arranged in the graphs from bottom to

top in the order of increasing relative growth-rates, i. e. increasing slope of the logarithm curves.

TIME-TABLE.

	Flowers tagged on.	Bolls collected on.	Age in days at collection.
Series A.	March 22, 1928.	March 29, 1928.	7
	" 23, "	" 28, "	5
	" 24, "	" 27, "	3
	" 25, "	" 26, "	1
Series B.	March 30, 1928.	April 5, 1928.	6
	" 31, "	" 4, "	4
	April 1, "	" 3, "	2
	" 2, "	" 2, "	0

To consider first the results for the normal ovules. Nitrogen content at first increases relatively more rapidly than does dry weight, but after the third day the rate of increase falls off rapidly as compared with dry weight. The average rate of increase of ash is similar to that of nitrogen, but it does not show the same high values during the first few days. Phosphorus shows the same high rate in the first few days as nitrogen, but its average rate is less. Calcium shows practically no increase for the first few days and has also the lowest average rate of increase.

The effect of fertilization is apparent in increased uptake, from the fourth day onwards, of water, dry weight, nitrogen, ash, and phosphorus. There is also some indication of an even earlier effect on phosphorus uptake. In the case of calcium uptake, on the other hand, there is little sign of any effect of fertilization until the 6th day. Owing to the small amount of material available potassium determinations could not be made on the ovules. We have suggested (22) that fertilization might (1) increase the rate of utilization of mobile compounds entering the boll from the phloem, and so steepen the gradient of entry, and (2) increase the ease of entry for these compounds. In both cases rate of uptake would increase. But for those compounds that move mainly in the transpiration current we might expect a much smaller effect of fertilization on uptake. It will be evident that the results obtained in this experiment for phosphorus uptake as compared with calcium uptake by the ovules are in accord with that expectation.

In the carpels, the divergence between calcium and the other ash elements, together with carbohydrates and nitrogen, is even more striking. To take the materials in turn, net water uptake is initially more rapid than dry weight uptake, but, after the 3rd day, the two curves for the normal bolls become parallel. Net water uptake is, however, increased much more than dry weight uptake by fertilization. As in the ovule the rates of increase of nitrogen and of phosphorus are initially much greater than the rate of increase of dry weight, but fall off later. The effect of fertilization on uptake of nitrogen and of phosphorus is very similar to the effect on

dry weight uptake. The uptake of ash is throughout very similar to the uptake of dry weight, but the effect of fertilization is rather less. Potassium uptake shows the highest average rate and the greatest effect of fertili-

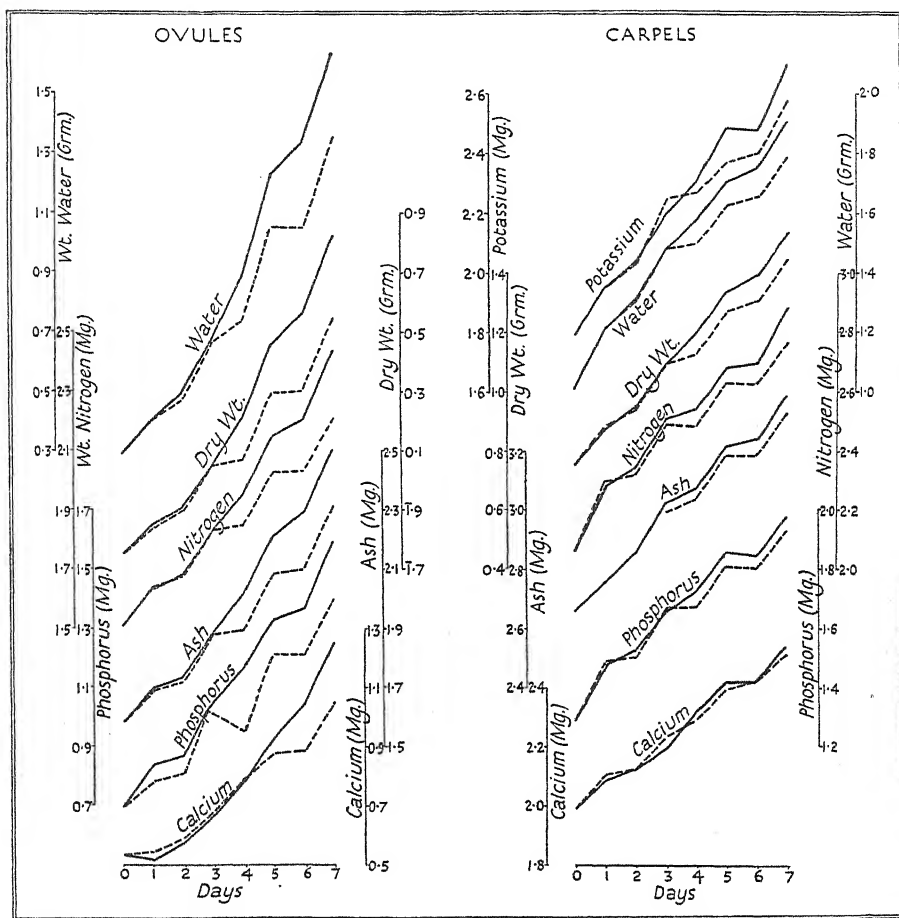


FIG. 3. Uptake of materials by *ovules* and *carpels* in normal and in unfertilized bolls during the first seven days from flowering. The results are plotted as \log_{10} of the weights per 100 bolls. Normal bolls, continuous lines: unfertilized, broken lines.

zation. The average rate of uptake of calcium is the lowest observed, and no effect of fertilization can be distinguished. The fact that there should be a small effect of fertilization on calcium uptake by the ovule but no appreciable effect on calcium uptake by the carpel is doubtless due to the fact that the amount of water lost by transpiration from the carpel may be very large compared with the amount of water retained for growth of carpel tissues; whereas relatively more of the water entering the growing ovule must be retained there, a smaller proportion being transpired from

the ovule surface (into the interior of the boll) than from the surface of the carpel. In consequence the greater net uptake of water by the normal as compared with the unfertilized ovule may involve an appreciably greater uptake of calcium carried in the tracheal sap, while the greater net uptake of water by the normal carpel may form so small a fraction of total intake of water that very little effect on total uptake of calcium results. It is possible also that the smaller net gain in moisture by the unfertilized carpel is due rather to a greater rate of loss by transpiration than to a smaller total uptake from the transpiration current. This might account for the observed identity of calcium content in normal and unfertilized carpels.

The differences in behaviour of different elements, observed in this experiment, must of course be confirmed and extended by further work. But in view of the interest of this method of attack on the problem of transport, and of the differences observed, it seemed desirable to draw attention to the results.

We suggested above that, in the rapidly growing ovule, the net uptake of water should be of the same order as the gross intake of water from the xylem, not much being lost by transpiration into the interior of the boll. (It is not, of course, certain that any is lost in this way, but it seems likely that the ovule will have a higher rate of respiration and, in consequence, a somewhat higher temperature than the carpel wall.) If this suggestion is correct, then a comparison of the ratios of the growth increments of different materials to the growth increments of water provides us with a test of the possibility that any one of them is imported mainly via the xylem; for the ratio of the increment of material to the growth increment of water will be an estimate of the concentration required in the incoming tracheal sap.

The ratios of the increments of carbohydrates, ash, phosphorus, and calcium to the net increment of water during the same (two-day) period are shown in Fig. 4. Carbohydrate is calculated as Dry weight-5.7 N-Ash. All materials are given as gm. per 100 gm. water. The results are, of course, those for the normal group of bolls.

Taking the ovules first, it will be seen that the rate of arrival of carbohydrate is roughly proportional to the rate of arrival of water. We cannot, however, entertain the suggestion that carbohydrates enter the ovule via the wood, since this would require a concentration in the tracheal sap of over 10 per cent., whereas the concentrations observed (p. 149) are of the order of 0.05 per cent. or less. The parallelism between carbohydrate and water uptake is doubtless due to the incoming sugars forming hydratable material, and so determining water uptake. The estimates of incoming concentrations for nitrogen, ash, and phosphorus show a well-defined trend, diminishing considerably as development proceeds. Moreover, even at the lowest points on the curves, the required concentrations are enormously in

excess of the concentrations found in the tracheal sap (Table IX). The figures are: minimum requirements for uptake by the ovule = nitrogen,

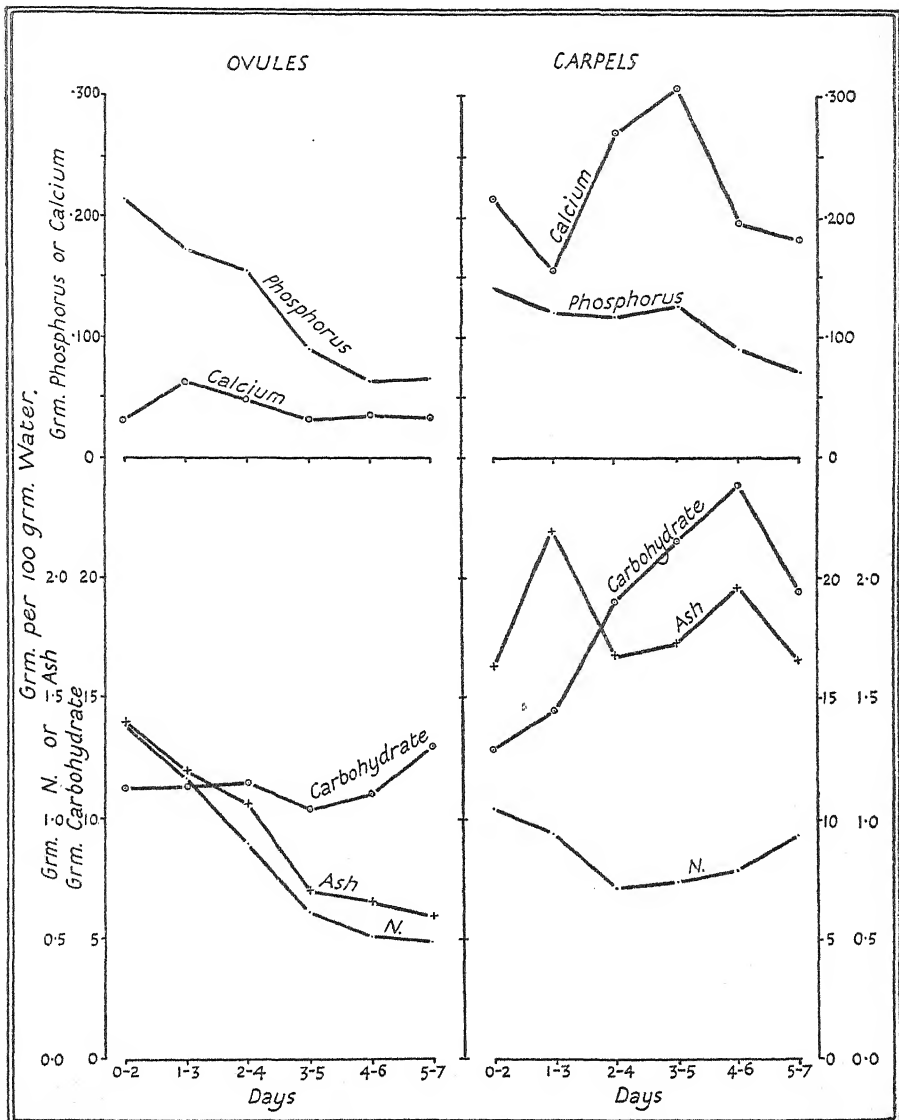


FIG. 4. Ratios of the two-day growth increments of carbohydrate, nitrogen, ash, phosphorus, and calcium, to the growth increments of water for the same periods, in ovules and in carpels of normal bolls. The ratios are plotted as concentrations (gm. per 100 gm. water).

0.49 per cent.; ash, 0.59 per cent.; phosphorus, 0.063 per cent.; concentrations in tracheal sap (p. 149) = nitrogen, 0.012 per cent.; phosphorus, 0.0039 per cent. (Figures for ash content were not obtained in the

tracheal sap, but, subtracting from the value for total solids the appropriate values for the other materials estimated at the same time (Table IX), the ash concentration could not be higher than about 0.05 per cent.) With even the minimum requirements so many times in excess of the probable concentrations in the tracheal sap it is clear that nitrogen, phosphorus, and most of the other ash constituents must enter the ovule via the phloem. The situation is rather different for calcium. The rate of arrival is very nearly proportional to the rate of arrival of water, and although the average concentration required (0.041 per cent.) is in excess of the concentration (0.0116 per cent.) found in the tracheal sap this difference is very small indeed when compared with the differences for phosphorus and for ash. While, therefore, uptake mainly or exclusively via the xylem seems to be definitely ruled out for phosphorus and for total ash, as well as for nitrogen and carbohydrate, it may possibly be true for calcium.

Even if the concentration in the tracheal sap supplied to the ovule were no higher than we found in the stem, an elimination of water, by transpiration into the boll cavity, equal to two and a half times the net uptake of water, would account for the observed uptake of calcium.

It must be noted, however, that the concentration of calcium already present in the ovule on the day of fertilization (day 0) is much higher than the average concentration entering subsequently, viz. 0.178 per cent. as against 0.041 per cent. The arrival of calcium in the ovule before fertilization would thus require much more extensive elimination of water. The rate of growth during this earlier growth-phase may, however, have been slow enough to permit of this.

Comparison of the ovule figures with those obtained for the carpels supports the suggestion as to calcium uptake outlined above. Since transpiration proceeds unchecked from the carpel surface, materials carried mainly or exclusively in the transpiration current should show in the carpels, as compared with the ovules, a marked increase in the ratio of increment of material to net increment of water. It will be seen from Fig. 4 that this is the case for calcium. Carbohydrate, nitrogen, phosphorus, and ash on the other hand, which travel mainly in the phloem, show a much smaller increase. Putting the mean values for the ovules equal to 1, the mean values for the carpels are: carbohydrate 1.62, nitrogen 1.02, ash 1.92, phosphorus 0.87, and calcium 5.35. If the bulk of the calcium in the carpels arrives via the transpiration current, some also of the ash, phosphorus, potassium, nitrogen, and carbohydrate may also arrive in the same way, since these substances are all present in the tracheal sap. In fact, if we knew the relative amounts of these substances in the tracheal sap of the boll pedicel we might, assuming that all the calcium had entered via the xylem, calculate what proportion of the total uptake of the other materials could have occurred via the wood. As an illustration

of the possibilities, let us take the following figures for the tracheal sap, representing roughly the maxima we have observed in the stem: total sugars 0.050 per cent., nitrogen 0.012 per cent., phosphorus 0.004 per cent., calcium 0.012 per cent. On this basis, if the xylem were responsible for the whole of the calcium uptake, it would account also for the following fractions of the total uptake of other materials: carbohydrate 5 per cent., nitrogen 25 per cent., phosphorus 66 per cent. These figures are, of course, very hypothetical, but the order obtained for the different materials is interesting, and in any case it is clear that, in the case of a growing organ which is also transpiring actively, an appreciable fraction of the total uptake of ash constituents and of nitrogen may take place via the xylem.

SECTION VI. THE MOBILITIES OF DIFFERENT ELEMENTS WITHIN THE PLANT.

(a) *Discussion.*

The results obtained in the ringing experiments have been summarized and their interpretation discussed in a previous section. Their relation to other investigations on the upward transport of mineral nutrients and to the general problem of the connexion between transpiration and the uptake of mineral nutrients from the soil has also been discussed.

In the observations on movement to the boll we found confirmation for the conclusion that, whereas nitrogen and phosphorus, and probably also potassium and some other ash constituents, are re-exported from the leaves, yet calcium is not re-exported in appreciable quantities from the leaf, and shows no definite evidence of movement via the phloem. This difference between calcium on the one hand, and nitrogen, phosphorus, and potassium on the other, raises the question of the mobility of different elements within the plant, concerning which a considerable amount of work has been done. Thus André (3), studying the movement of materials out of the cotyledons in young seedlings of 'haricots blancs' germinating in the dark, found that the elements were mobilized in the following order: nitrogen, phosphorus, sulphur, potassium, magnesium, calcium. In the case of calcium 66 per cent. remained in the cotyledons, while in the case of nitrogen and phosphorus there was only 27-28 per cent. left. Again Swart (31) found in leaves just before leaf-fall a decrease of nitrogen and potassium which could only be explained by a migration into the axis. Magnesium, iron, calcium, silicon, sulphur, and chlorine, on the other hand, showed little or no change.

The distribution of the elements in the different parts of the plant also suggests that some of them accumulate in, and are remobilized with difficulty from, the tissues which they reach via the transpiration current. We have already drawn attention (p. 154) to the different distribution, in

leaves, bark, and wood, of calcium as compared with phosphorus and potassium. If we compare the leaf with the seed the differences are even more striking. In Table XIV the weights of different elements per 100 gm. dry weight in the seed of cotton are expressed as a percentage of the corresponding weights, per 100 gm. dry weight, in the leaf. The data are taken from Brown (5).

TABLE XIV.

Weights of elements per 100 gm. dry weight in the cotton seed expressed as a per cent. of the corresponding values for the leaf.

Nitrogen	157
Phosphorus	292
Potassium	103
Sodium	42.4
Magnesium	31.9
Sulphur	10.5
Iron	7.0
Calcium	6.1
Silica	1.2

In the leaf the mineral elements must normally arrive via the transpiration current, and apart from possible loss by the leaching action of rain any that are not re-exported via the phloem must accumulate. In the seed the bulk of the material must come via the phloem, and consequently only those materials which are re-exported from the leaf via the phloem can arrive there in any quantity. Some difference must, of course, be ascribed to the different nature of the living cells in leaf and seed, but it is difficult to resist the conclusion that the striking differences shown in Table XIV are very largely the result of differences in the case of re-mobilization from the leaves of the elements that reach them via the transpiration current.

There seem to be differences also in the capacity for remobilization of elements accumulated in the older parts of the plant. Thus Reed and Haas (28) report that orange trees grown in cultures deficient in calcium seemed to be unable to utilize for new growth the calcium present in the trunk and root. Similarly Amar (1) found, in plants transferred to calcium free solutions, no diminution of the calcium oxalate crystals in the lower leaves, although the development of new leaves was hindered by the lack of calcium. With other elements redistribution from the older tissues to the growing regions as supply falls off, or is curtailed, seems to be a regular feature. For nitrogen the effect has long been known, while MacGillivray (17) has recently established it very clearly for phosphorus in the tomato plant. When the supply of phosphorus was cut off at an early stage in growth about 70 per cent. of the phosphorus in the leaves and stem of the lower region moved up to the growing regions. A similar redistribution

has been claimed for potassium by Janssen and Bartholomew (16), but, owing to some doubt as to the completeness of curtailment of supply, their evidence is not conclusive. Again, Gregory and Richards (12) report for barley, grown in sand cultures deficient in potassium, that potassium liberated by the older parts of the plant is translocated up to the later formed leaves.

As to the path by which this remobilization of materials takes place and the conditions determining the direction of transport, we have as yet no information. In view of our conclusion that the nutrient materials that move down the phloem may also leak into the tracheae and again pass upwards, it would seem that both xylem and phloem might play a part in the upward translocation of stored material. But transport along gradients in the phloem must presumably play the chief part in satisfying the nutrient hunger of the growing tissues. Otherwise growing organs that were not actively transpiring might fail to obtain adequate supplies. Experiments on cotton are in progress which will, we hope, throw some light on this question.

The reason for the comparative immobility within the plant of an element like calcium, that is essential for growth, is likely to prove a much more difficult problem. From the results of the experiments described in this paper it seems clear that the immobility of calcium within the plant is connected with its inability to move in the phloem. Calcium oxalate crystals are abundant in the ray cells of the phloem, but we have not yet been able to satisfy ourselves by micro-chemical tests of the presence of calcium in the sieve tubes. It may be that calcium cannot gain access to the conducting cells. It seems, however, on the whole, more probable that calcium may gain access via the transpiration or the growth water current to all cells, but that once within the cell it is either precipitated or combined with tissue material in such a way that very few free calcium ions are left in solution. If the equilibrium point is sufficiently far over on the side of combined calcium, there would be little tendency for the cell to give up its calcium to an external solution or to a neighbouring cell poor in calcium. It is difficult otherwise to understand why the calcium present in the older regions of the wood should not be liberated into the transpiration stream, when this becomes poor in calcium, and so migrate to the developing leaves. It should be said, however, that in analysing the sap expressed, after freezing, from a sample of bark we found nearly half as many mg. equivalents of calcium as of potassium in the boiled and filtered sap. If this is to be interpreted as indicating the existence, within the living cells of the bark, of a considerable concentration of calcium in free solution, then a relative impermeability of the cell membranes for outward passage of calcium may be the explanation of the immobility of calcium. The positions occupied by potassium and calcium respectively in the lyotropic

series make this explanation of their different behaviour in the plant not unlikely. As, however, no study of the concentrations of calcium in the sap expressed from other parts of the cotton plant has yet been made, nothing is known about the gradients of free calcium ions.

One further conclusion emerging from this discussion of the transport of calcium and of other relatively immobile but essential elements is that, since these elements are distributed through the plant mainly in the movement of transpiration or growth water, curtailment of supply in the solution bathing the roots should very soon lead to disturbance and cessation of growth. Gericke (11) has, in fact, found that the elements required longest for normal development of wheat are calcium and iron. Nitrogen, phosphorus, and potassium, however, may be curtailed at a very early stage without causing a cessation of growth. The continued need for calcium (cf. 13, 14) may of course be partly due to the necessity (cf. 10) at all times of free calcium ions in the external solution bathing the roots.

Something should be said, in conclusion, as to the bearing of the results obtained for transport of ash materials on the general problem of phloem transport. It will be noted that, under the experimental conditions chosen, using plants in an active vegetative condition growing in the open, upward transport of nutrients absorbed by the roots has occurred via the xylem; so that we have not, as might have been anticipated (cf. p. 127), obtained evidence of the upward movement of materials along a region of phloem which is at the same time serving for the downward movement of assimilates. If there was some upward movement of inorganic materials via the bark as well as via the wood, it was, under the experimental conditions, completely overshadowed by the upward movement via the wood, and the re-export and downward movement, possibly in organic form, via the phloem. Clearly, however, the results do not exclude the phloem as a possible path of upward transport, and other experimental conditions must also be tested. Of these we may mention (1) a study of the upward redistribution, following curtailment of supply to the roots, of nitrogen and other nutrients stored in the lower region of the plant, (2) a study of the upward movement of nutrients under conditions of high humidity and increased nutrient supply. Under these conditions carbohydrates should continue to move downward via the phloem, while there might be appreciable upward movement of nitrogen and other nutrients via the phloem as well as via the wood. Work with cotton plants along these lines is now in progress.

(b) SUMMARY.

1. When a ring of bark is removed from the main axis of cotton between the foliage region and the roots, phosphorus, potassium, and possibly some other ash constituents, as well as carbohydrates and nitrogen,

accumulate in the stem tissues above the ring and diminish below. There is also a significant accumulation of total ash as well as of nitrogen and carbohydrates in the leaves above the ring.

2. The results obtained indicate that nitrogen, phosphorus, potassium, and other ash constituents ascend the stem mainly via the wood, are re-exported from the foliage, and move downwards towards the roots via the phloem. Calcium also ascends via the wood, but there is no evidence that it is re-exported from the leaf to move downwards via the phloem.

3. This downward movement of phosphorus and of ash may, like the parallel movement of nitrogen and of carbohydrates, be reversed by reversing the relative positions of leafy and leafless regions of stem. Data were not obtained for potassium.

4. The ratios of nitrogen, phosphorus, and potassium in downward movement to the carbohydrate in downward movement appear to be in excess of the ratios required for growth of the lower part of the plant. It is suggested that the excess may be liberated into the tracheal sap, and so again ascend the plant. This suggestion is supported by the composition of the tracheal sap, notably its low content of sugars as compared with mineral materials, and also the fact that as much as one-third of the nitrogen in the tracheal sap may be organic. It is pointed out that this return towards the roots of many of the mineral nutrients that have moved upward with the transpiration current must influence uptake by the roots from the external solution, and may in part explain why the effect of increasing transpiration rate on rate of salt uptake is so comparatively small.

5. The relative concentrations, in wood, bark, and leaf tissues, of calcium, phosphorus, and potassium are in harmony with the view that phosphorus and potassium may be readily re-exported from the tissues they reach via the transpiration current, while calcium is re-exported much more slowly, if at all.

6. Removal of the growing bolls is followed by a marked increase in the concentrations of phosphorus and total ash as well as of nitrogen and carbohydrates in the leaves and stem tissues of the plant. Calcium also increases, but to a smaller extent.

7. Fertilization markedly increases the rate of uptake of phosphorus and total ash, as well as of carbohydrates and nitrogen, by the ovule, but has a smaller effect on the uptake of calcium. There is a similar effect in the carpels, but the divergence between the effect on calcium uptake and that on the uptake of total ash, phosphorus, and potassium is even greater than in the ovule. The results are in harmony with the view that phosphorus, potassium, and some other ash constituents travel to the boll along gradients in the phloem, while calcium moves mainly in the xylem.

8. Assuming that the rate at which water enters the young ovule, which is growing rapidly and is largely protected from transpiration, is of

the same order as the rate of net gain in water, then the ratio of the increment of any material to the net increment of water should be an estimate of the order of concentration required in the incoming tracheal sap, if import were entirely via the xylem. For calcium the concentration so calculated is roughly of the order observed in the tracheal sap of the stem; for nitrogen, carbohydrates, total ash, and phosphorus, the concentrations indicated are vastly in excess of those found in the tracheal sap.

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Water Conduction in *Polytrichum commune*.

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With Plate VII and seven Figures in the Text.

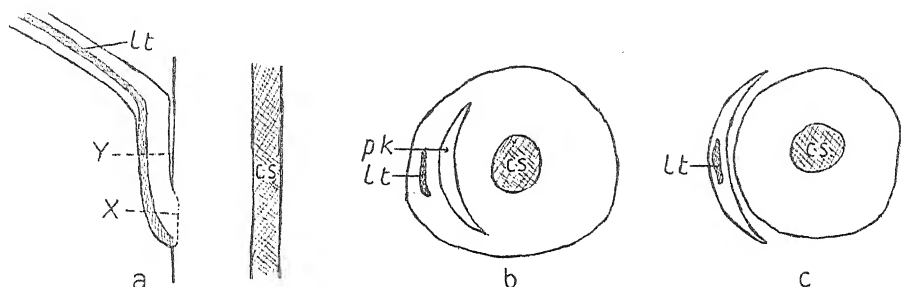
SINCE the publications of Haberlandt (6) in 1886, Bastit (1) in 1891 followed by Coesfeld (3) in the following year, no noteworthy investigation has been made on the channel of water conduction in mosses. It is generally recognized that in the Sphagnales water is conducted not internally in the stem, but either as capillary films between the stems and the pendent branches, or in the outer enlarged cells of the cortex. In the higher mosses, however, very little information is available. The work of Tansley and Chick (8) in 1901 on *Polytrichum commune* was confined entirely to anatomical details, and while a careful examination was made of the structure of the so-called conducting strand of this moss, actual physiological experiments were largely omitted. It has therefore been assumed very generally that the work of water conduction in *P. commune* is carried out entirely by the central strand, an assumption which, it appears, is by no means fully justified.

The fact had long been noticed that the angle of divergence of the leaves of the moss plant from the stem varies a good deal with the habit of the plant (whether it is growing as an isolated individual or in densely-packed clumps) and with its habitat (whether a dry or a wet one). It seemed likely that the situation of the leaf in relation to the stem might have a direct bearing on the power of the plant to conduct water externally, and this matter is under investigation for a large number of mosses. A good deal of preliminary work was done, however, on *P. commune* with a view to determining whether external conduction does take place in this plant. The result of this work is the subject-matter of the present report.

The leaves of *P. commune* are furnished with long and broad leaf-bases composed of a single layer of cells, except in the region of the midrib. In the lower region the sides of these leaf-bases are adherent to the stem, forming small pockets which can conveniently hold water. Transverse sections of the stem taken near the base of the leaf show the internal surface of the pocket bounded by the epidermis of the stem and the adaxial

surface of the leaf-base, while sections higher up show the leaf-bases detached from the stem leaving a narrow channel between the two (Text-fig. 1).

The arrangement of the leaves on the stem with a spiral phyllotaxy is such that a complete, external spiral connexion exists between one pocket



TEXT-FIG. 1. (a) Diagram of longitudinal section through stem and leaf. (b) Diagram of transverse section in region of X. (c) Diagram of transverse section in region of Y. (Lt. = leaf-trace; pk. = pocket between leaf and stem; cs. = central strand.)

and the next, from the base to the tip of the *Polytrichum* plants, through the narrow channels. Moreover, the width of the channels between the leaves and the stem varies with the amount of water available. Under normal conditions, i.e. when transpiration is slow (for rarely do these plants grow in areas exposed to either wind or direct sunlight), the leaves stand off at an angle of about 45 degrees from the stem and the channel is comparatively wide (Pl. VII, Fig. 1). If these plants are subjected to conditions expediting transpiration the leaves take on a wilted appearance, the lateral portions of the leaf-blade bending upwards and enveloping the assimilating lamellae, enclosing them in a hollow chamber. At the same time the leaf becomes more closely approximated to the stem, thus narrowing the channel between leaf and stem, and therefore retaining more effectively any water held there by capillary action (Pl. VII, Fig. 3). On the other hand, in a saturated atmosphere the reverse process takes place, and the leaves bend backwards in such a way that any water falling on their surfaces trickles towards the tips and so rolls off (Pl. VII, Fig. 2). These leaves have thus adopted the same principle as the drip-tips of some hygrophilous angiosperms. This fact was proved very clearly by placing some growing plants of *Polytrichum* in a pot under a bell-jar together with a beaker of boiling water, a sheet of asbestos screening the plants from the heat rays radiated from the beaker. The steam saturated the atmosphere and condensed on the leaves, with the result that, in a very short time, they began to bend backwards from the stem, this bending becoming more and more pronounced until they had assumed almost a pendent position. They retained this position long after the contents of the bell-jar had

reached room temperature; and only after the bell-jar was removed, and the plants were therefore allowed to dry off in the atmosphere, did they resume their normal position with respect to the stem.

EXPERIMENTAL.

A number of experiments were carried out in order to determine whether any external rise of water does take place in the moss plant and, if so, the rate at which the rise occurs and the extent to which such rise is sufficient to satisfy the needs of the plant.

(A) *Use of Lycopodium Powder.*

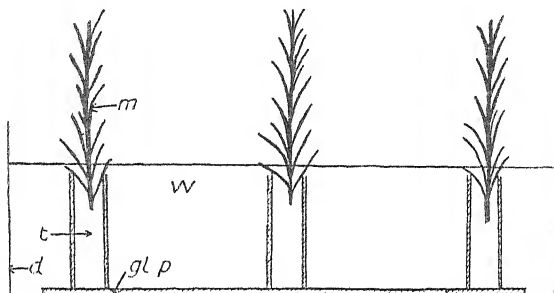
Polytrichum plants were thoroughly washed and left to dry in the air until all traces of external moisture had disappeared. (Plants so treated will be referred to hereafter as 'air-dried' for the sake of brevity.) They were then placed in bowls of water, the surface of which was dusted over with *Lycopodium* powder. On examining the plants with the binocular microscope the powder was seen to be rising on the surface of the plant, the rise at first being very rapid but slowing down later, apparently owing to the tendency of the particles of powder to accumulate in the axils of the leaves and block further ascent, though the rise of water could be seen far above the level of the powder. This simple initial experiment proved clearly that there is a fairly rapid rise of water over the external surface of the plant when it is in contact with a plentiful source below.

(B) *Wilting Method.*

Following to some extent the method of Dixon (5), for blocking angiosperm vessels and tracheides, the cut ends of plants were carefully dried and placed for 30 minutes into melted paraffin wax which was kept molten over a warm water bath, or in an oven, at a temperature of 50° C, the melting-point of the wax being 45° C. The plants were then submerged in cold water for five minutes to harden the wax, and were then allowed to dry off until they were decidedly wilted in appearance before being placed in bowls of water. In order to prevent any external deposit of water on the plants, it was obviously necessary to isolate them from one another, and to ensure that they did not touch the sides of the bowl containing the water. For this purpose small stands, constructed in the following way, were used. Pieces of glass tubing 3 cm. long and 0.7 cm. in diameter were cemented on to glass plates 8 cm. x 5 cm. at an average distance of 3 cm. apart. The stands were placed in dishes of water, the level of which reached about 0.5 cm. above the top edge of the glass tubes. A single *Polytrichum* plant was then inserted into each tube (Text-fig. 2).

It was observed that the leaves gradually regained their freshness and

stood out from the surface of the stem, those nearer the surface of the water showing this effect first, and the effect extending gradually up the plant. Since the ends of the stem were blocked with wax it is clear that sufficient water must have travelled up over the external surface of the plant to revive the freshened leaves. As a means of measuring accurately the rate of ascent, this method is obviously useless, but it is of value as confirming the occurrence of an external rise.



TEXT-FIG. 2. Diagram of apparatus used. (*d.* = dish; *t.* = glass tube; *gl. p.* = glass plate; *w.* = water; *m.* = moss).

A further experiment was made in which two series of plants, one series with the cut ends of the stems blocked with wax, the other series of normal complete plants, were planted in soil and left for one month in a cold frame. At the end of this time there was no visible difference in the freshness of the plants of the two series, proving that the external conduction in the first series was sufficient to maintain the plants in their normal healthy condition.

(C) *Colour Reaction Methods.*

1. The cut ends of stems were blocked with wax as described above and the air-dried plants were dusted all over with finely powdered starch. They were then placed in stands in bowls of a dilute solution of iodine in potassium iodide. The colour of the starch gradually changed to purple, the change proceeding from below upwards, suggesting that the iodine solution was moving up over the external surface. However, a check experiment in which the plants dusted with starch were placed not in the solution, but above it, showed that the vaporization of the iodine was sufficient to turn the starch purple, though the rate of this change was considerably slower than the rate when the plants were dipping into the iodine solution. This vaporization was diminished by adding a higher proportion of potassium iodide, but the necessary concentration caused a wilting of the leaves, due to exosmosis, and therefore the method had to be abandoned, though it afforded some confirmation of the occurrence of an external rise.

A further attempt was made to use a colour reaction to determine the rate of rise.

2. To an alcoholic solution of phenolphthalein was added sufficient solution of sodium hydroxide to give a deep red colour. Starch powder was moistened with this liquid, forming a pink paste. The mixture was then left to dry and finely powdered. The pink powder was dusted over *Polytrichum* plants prepared as described under (C) 1, and then placed in stands in dishes containing centinormal sulphuric acid solution. As the liquid rose over the moss stem the colour of the powder changed from pink to white, and the rate of rise could be determined by the rate of change. Accurate readings were obtained by the use of the reading microscope, and the height of ascent at varying intervals in a number of plants are shown in the following table :

TABLE I.

Plant No.	Height in cm. to which acid had risen in				
	1 hr.	2 hrs.	3 hrs.	4 hrs.	24 hrs.
1	3.6	3.8	3.8	—	4.3 (tip)
2	3.6	3.6	4.3	—	5.0 "
3	3.4	3.8	4.3	—	4.8 "
4	1.8	2.3	2.8	—	3.3 "
5	2.5	2.8	3.0	3.5	3.8 "
6	4.0	5.0	5.2	5.8	6.1 "
7	4.9	5.0 (tip)	tip	tip	tip "
8	2.8	2.8	3.0	3.4	3.8 "
9	4.1	4.2	4.5	4.7	4.8 "
10	1.9	2.8	3.3	3.6	3.9 "

In order to determine whether either the powder or the acid had any deleterious effect, three series of plants were prepared for experiment :

1. Plants blocked with wax and placed in dishes of water only.
2. Plants blocked with wax, dusted with powder, and placed in dishes of water.
3. Plants blocked with wax, dusted with powder, and placed in dishes of acid.

All the dishes were placed separately under bell-jars and left for a week, at the end of which time the powder on the plants in 3 had become pink. There was, however, no visible difference in the three series as far as the healthy appearance of the plant was concerned, indicating that the reagents employed had had no harmful effect. Moreover, the colour of the powder on 2 had not changed at all, proving that the change in 3 was due to the rise of the acid solution on the exterior and not to plant acids diffusing out from the cell contents.

(D) *Use of Stains.*

Any external rise of liquid was clearly visible if the liquid were coloured. Different stains were therefore experimented with, the best

results being obtained with 0.5 per cent. solutions of eosin, safranin, and gentian violet. As a means of measuring the rate of rise, this method was excellent, for the coloured liquid could easily be seen accumulating in the leaf axils. Plants treated precisely as described in (C) were therefore placed in dishes of these stains, and the height of rise carefully noted from time to time. The following table gives a typical instance of the figures obtained:

TABLE II.

Plant No.	Height in cm. to which stain had risen in				
	1 hr.	2 hrs.	3 hrs.	5 hrs.	24 hrs.
1	5.84	5.88	6.0	—	6.5 (tip)
2	2.0	2.3	5.6	—	6.0 "
3	3.8	4.1	4.3	—	4.8 "
4	3.2	5.0	6.0	—	6.9 "
5	2.2	2.8	4.0	4.3	4.6
6	1.3	1.7	3.0	4.6	4.6 "
7	1.8	2.5	4.0	5.0	5.0 "
8	3.0	6.0	6.0	6.4	6.4 "
9	4.0	6.1	6.4	7.1	7.1 "
10	4.6	5.0	5.6	5.8	6.2 "

(E) *Use of Colloidal Solutions.*

Apart from the experiments with *Lycopodium* powder, there was still a possibility that, where true solutions were used, the solution might pass into the plant through the external walls, in spite of the block at the cut end, rise internally, and diffuse out again. An attempt was made to investigate the effect of colloidal solutions, which could not pass into the cells, and any accumulation of which on the plant would obviously be due to a rise externally. Arsenious sulphate was first tested, but it was practically invisible on the surface of the plant, and its use was therefore discontinued. A 0.5 per cent. solution of dialysed iron gave better results in that it proved to be much more easily identified. When plants were placed with their blocked cut ends in stands in dishes of this solution, it eventually rose to the tips of the plants, and drops of it could be seen at the tips by the aid of the reading microscope—a conclusive proof of external conduction. The heights to which this solution had risen on a number of plants during varying periods are shown in Table III.

As a means of measuring the rate of ascent this method was relatively useless, for some time elapsed after the solution had reached a definite point on the stem (as shown by careful microscopic examination at close quarters) before sufficient of it accumulated at that point to form a drop large enough to be visible *in situ* in the dish with the reading microscope. Its value lay entirely in the proof which it afforded of the external rise of liquids.

TABLE III.

Plant No.	Height in cm. to which dialysed iron had risen in				
	1 hr.	2 hrs.	3 hrs.	5 hrs.	24 hrs.
1	2.0	2.5	2.5	—	3.0
2	2.0	2.3	2.3	—	2.7
3	1.5	2.3	3.6	—	4.0
4	1.5	1.6	1.9	3.6	3.9
5	1.0	1.1	3.8	4.0	4.5
6	1.9	2.2	2.2	3.0	4.2
7	3.4	3.4	4.5	4.6	6.0
8	2.4	3.3	3.5	4.0	5.0
9	2.8	3.8	3.8	4.0	5.8
10	1.4	2.2	2.5	2.7	4.6

(F) *Spectroscopic Examination.*

The cut ends of stems were blocked with wax as previously described and the ends placed in stands in dishes of a 0.5 per cent. solution of lithium sulphate. At the end of varying intervals the plants were removed and the stems cut in pieces 5 mm. long. Each piece was incinerated on a piece of platinum wire in a bunsen flame, the flame being examined spectroscopically for the lithium line. Although the occurrence of lithium in the upper regions of the plant does not, in itself, afford a definite proof of external conduction, the method was useful in obtaining details of the rate of the ascent after short intervals.

Figures obtained are shown in the table below :

TABLE IV.

Plant No.	Total height of plant in cm.	Duration of experiment.	Extent of rise of lithium sulphate in cm.	Ratio of rise to total height of plant.
1	12	10 mins.	4.5	0.375
2	10.5	10 "	4.5	0.43
3	10	30 "	5.5	0.55
4	14	30 "	3.5	0.25
5	12	1 hr.	2.0	0.17
6	12	1 "	6.0	0.5
7	13.5	1 "	12	0.9
8	10	3 "	10	1

From the data given it is evident that conduction of liquids over the external surface of the moss plant takes place rapidly at first, but that the rate of ascent diminishes markedly after the first hour. This result would be expected were the rise due simply to capillarity, but the suggestion arose that osmotic attraction on the part of substances contained in the cells of the leaf-base might also play a part. Series of experiments were therefore set up in order to test the comparative rate of rise of external

water in (A) normal plants, and (B) plants whose leaf-axils had been provided with osmotic materials by placing small grains of cane-sugar in them. The following table is typical of the results obtained:

TABLE V.

Rise of gentian violet on plants (A) with ends blocked with wax, and (B) with ends blocked with wax and grains of sugar placed in the axils of the leaves.

Plant No.	Height in cm. to which stain had risen in													
	1 hr.		2 hrs.		3 hrs.		4 hrs.		5 hrs.		6 hrs.		24 hrs.	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	13.5	10	15.5	20	20	30	74.5	57.4	77.6	62.1	94.5	84.2	96	85
2	31.2	30	33	30	33	30	38.3	46.9	38.5	49.3	41.6	49.2	42.4	50.6
3	15.7	18.1	16	18	20.2	30	65.2	72.7	72	85.2	75	92	75	92
4	20.2	18	31.5	19	36.4	28	39.1	51.9	40.2	52	43	52	43.5	52.6
5	27	20	35	25.7	59.3	50.5	59.5	51.5	60.5	52	60.5	52.5	60.5	53
6	12.2	21	14.6	24.6	21.2	25	47.5	49	62	67	64	67	64	67
7	16	30.5	18.9	36.1	24.6	37	26.4	37.5	34.6	38	36.4	38	36.4	38
8	22.3	13	25.7	25	45	48.8	45.5	56	46	59.5	46	49.5	46	50
9	10.5	12.6	35.6	27.7	—	—	—	—	—	—	45.2	50	—	—
10	38.8	36.3	38.8	43.2	—	—	—	—	—	—	50	56.2	—	—
11	33.2	22.7	34	33	—	—	—	—	—	—	39	45	—	—
12	41.9	37.2	42	38	—	—	—	—	—	—	53.4	62	—	—

The variability of these results indicates that it must be concluded that capillarity is the chief factor concerned in the external conduction of liquids, though osmotic attraction by substances contained in the cells of the leaf-base and by any substances in the leaf-axils may play some part, especially in the upper regions of the plant, the effect varying considerably with the individual plants.

THE ELIMINATION OF THE CENTRAL STRAND AS A MEANS OF INTERNAL CONDUCTION.

On account of the general acceptance of the view that the central strand of *Polytrichum* is effective in conduction, it was deemed desirable to eliminate entirely any possibility of internal conduction by the destruction of this strand, and to test the rate of rise of water in plants so treated. A red-hot needle was therefore inserted into the cut ends of a number of strong *Polytrichum* plants, destroying the central tissues to a height of at least one centimetre. These plants were then placed in stands in solutions of lithium sulphate in the usual way. The rise of lithium was estimated spectroscopically, and the results of the examination are shown in the table following.

TABLE VI.

Plant No.	Height of plant in cm.	Time.	Rise of lithium sulphate in cm.	Ratio of rise to height of plant.
1	12	30 mins.	4	0.3
2	10	"	5	0.5
3	17	1 hr.	5	0.3
4	12	"	5	0.4
5	16.5	3 hrs.	13	0.8
6	13	"	13	1
7	10	"	9.5	0.95
8	13.5	4 hrs.	9.5	0.7
9	10	"	10	1
10	12	"	11	0.925
11	13.5	1 day	12	0.9
12	15	"	15	1

Plants similarly treated were also placed in dishes of a 0.5 per cent. solution of potassium nitrate. At intervals, sections were cut at varying levels from selected plants, and mounted in a drop of solution of diphenylamine in concentrated sulphuric acid. The height to which the liquid had risen in the given time was indicated by the highest level at which appeared the deep blue coloration due to the interaction of the nitrate with the diphenylamine. The results are shown in the table below:

TABLE VII.

Plant No.	Height of plant in cm.	Time.	Rise of potassium nitrate in cm.	Ratio of rise to height of plant.
1	11.5	1 hr.	9.5	0.83
2	10	"	9	0.9
3	18	"	12	0.7
4	9	4 hrs.	9	1
5	12	"	11.5	0.96
6	12	"	12	1
7	9.5	5 hrs.	9.5	1
8	18	"	17	0.95
9	13	"	13	1
10	12	24 hrs.	12	1

A comparison of these figures with those obtained for the rise of various materials on stems, the cut ends of which had been blocked with wax, shows that the results obtained by blocking the central strand and by destroying it are very similar. In both cases a marked external conduction of liquids takes place.

The continuity of the central strands in a number of plants was next interrupted by carefully cutting away the central tissue, for a distance of one centimetre, care being taken to prevent injury to the outer tissues and the leaf-bases on one-half of the circumference of each stem in the excised region. The central region was removed from different levels in the

various plants. The plants were placed in stands, some in solutions of lithium sulphate and some in potassium nitrate. The height of ascent of the liquid was investigated in the manner described above, and the results are shown in the following tables :

TABLE VIII.

Plant No.	Height of plant in cm.	Height of cut region in cm.	Time.	Rise of lithium sulphate in cm.
1	12	6-7	30 mins.	9
2	17	10-11	1 hr.	12
3	12.5	9-10	1 "	10.5
4	20	10-11	3 hrs.	19.5
5	8.5	5-6	4 "	8.5
6	11	8-9	18 "	10
7	13	5.5-6.5	18 "	13
8	16	9-10	24 "	15
9	10	6-7	24 "	10

TABLE IX.

Plant No.	Height of plant in cm.	Height of cut region in cm.	Time.	Rise of potassium nitrate in cm.
1	21.5	10-11	1 day	21.5
2	15	8-9	"	15
3	18.7	10-11	"	15.5
4	12	9-10	"	11.5
5	15	6-7	"	10
6	16	8-9	"	16

It is evident from these figures that the removal of the central tissue did not appreciably interfere with the ascent of water, and that conduction of water must take place over the external surface of *Polytrichum* plants, extending to the tips of the plants, and independently of any internal conduction.

DETERMINATION OF THE TOTAL AMOUNT OF LIQUID CONDUCTED EXTERNALLY.

In order to determine the amount of liquid externally conducted, an initial experiment was performed in which some complete *Polytrichum* plants and some plants with cut stems, their cut ends blocked with wax, were placed in stands, as above, and left for one day in a 0.5 per cent. solution of sodium chloride. At the end of a day a white deposit was visible at the tip of the plant (Pl. VII, Figs. 4 and 5), and it was evident that if the amount of this deposit could be estimated a measure could be obtained of the volume of liquid which had travelled up to cause it. This experiment was repeated with large numbers of plants. At the end of varying intervals the plants were removed from the salt solution and washed carefully and thoroughly. The total washings obtained from each plant were titrated

against a centinormal solution of silver nitrate, potassium chromate being the indicator used. In this way the amount of sodium chloride which had risen on the plant surface was estimated, and since the original strength of the solution in the dish was known the amount of liquid which had passed up over the external surface could be calculated.

Estimations were made of three categories of plants:

(A) Complete plants which were submerged to the level of the living green leaves.

(B) Complete plants which were submerged to the level of the dead brown leaves.

(C) Cut stems whose ends cut to the level of the green leaves had been blocked with wax.

The following table gives the comparative results obtained:

TABLE X.

Time.	A.		B.		C.	
	Height of plant in cm.	Vol. of liquid passed up in c.c.	Height of plant in cm.	Vol. of liquid passed up in c.c.	Height of plant in cm.	Vol. of liquid passed up in c.c.
1 day	10.5	1.84	10.5	1.76	—	—
1 "	—	—	10	1.43	—	—
2 days	10.2	0.36	17.3	2.16	9.4	0.965
2 "	11.9	2.81	11.3	3.16	—	—
2 "	—	—	10.6	0.18	—	—
3 "	4.8	0.789	25.8	9.37	14.5	5.79
3 "	9.6	1.58	26.4	2.8	—	—
3 "	7.6	1.46	19.6	4.15	—	—
4 "	9.1	3.4	21.6	9.3	11.2	3.7
4 "	6.5	1.37	18.3	1.52	—	—
5 "	12.86	8.54	29.2	9.65	9.1	2.8
7 "	8.4	5.06	13.2	0.82	—	—
7 "	8.13	4.59	12.2	0.69	—	—

A glance at this table shows that an appreciable amount of liquid, as measured by the actual deposit of salt, had passed to the tips of the plants by the end of the first day. It might reasonably be expected that the volumes of liquid rising over the surface of the plants during successive days would be directly proportional to the duration of the experiment. It is evident that the data given do not fulfil this expectation, and therefore the method of measuring the volume of ascent by the total accumulation of salt is most probably inaccurate, since the salt deposit becomes so marked by the end of a day that it is almost inevitable that small particles from the deposits on the leaves will constantly fall back into the dish below. This suggestion will account for the fact that in almost all cases the amount of liquid passed up, as measured by the amount of deposit, is actually less at the end of the seventh day than at the end of the fifth—a condition which obviously could not have prevailed.

The following experiments were therefore performed in order to test the validity of the suggestion :

Experiment 1. Three series of *Polytrichum* plants were placed in stands in dishes of sodium chloride and left for one week, when the salt deposit was washed off each plant and titrated as before. The three series were as follows :

- (A) Complete plants submerged to the level of the rhizome.
- (B) Complete plants submerged to the level of the brown, dead leaves.
- (C) Complete plants submerged to the level of the green, living leaves.

The figures obtained at the end of the week were as follows :

TABLE XI.

Time.	A.		B.		C.	
	Height of plant in cm.	Vol. of liquid passed up in c.c.	Height of plant in cm.	Vol. of liquid passed up in c.c.	Height of plant in cm.	Vol. of liquid passed up in c.c.
7 days	28	1.3	33.8	4.85	8	1.99
7 "	8.4	5.1	25	4.3	10	2.7
7 "	30	1.5	28	5.3	12	2.3

Experiment 2. At the same time three series of plants (A, B, C, as above) were placed in sodium chloride solutions for a week, but each plant was washed at the end of the first, second, third, fifth, and seventh days and then replaced in the salt solution. The total washings from each plant for the week were accumulated and titrated as before. From the figures obtained the volumes of liquid which must have risen over the respective plants were calculated, and are shown in the table below :

TABLE XII.

Time in days.	A.		B.		C.	
	Height of plant in cm.	Vol. of liquid passed up in c.c.	Height of plant in cm.	Vol. of liquid passed up in c.c.	Height of plant in cm.	Vol. of liquid passed up in c.c.
1st	24	0.4	28.6	1.0	8.4	0.64
2nd	"	0.53	"	1.17	"	0.87
3rd	"	0.64	"	2.52	"	1.58
5th	"	0.523	"	1.58	"	1.29
7th	"	0.7	"	1.87	"	1.46

From Table X it is seen that there is an increase in the conducting capacity for the first three days, while the total for the fourth and fifth days is less than for the third day alone, so that the rate of rise evidently diminishes gradually after the third day. This may be due to a deleterious effect of the salt solution after four days, diminishing the metabolic activity of the plant and therefore its need for water. In spite of this diminution in

the rate of ascent during the later stages of the experiment, however, it is interesting to note that a considerable volume of liquid—considerable in proportion to the size of the plant—has passed up over its external surface in the course of a week, this volume, in one case, amounting to more than 8 c.c.

The conclusion reached, as a result of the experimental work so far reported, is that there is a fairly rapid rise of water to the tips of *Polytrichum* plants over the external surface of the stems, and that the amount of water so conducted is large and sufficient to maintain the plant in a healthy condition.

INTERNAL STRUCTURE OF *POLYTRICHUM COMMUNE*.

The question naturally arose as to where the entry of water takes place, and what is its internal path, for entry into the interior must take place in order that the internal tissues may be supplied. Attention was therefore turned to the internal structure.

Haberlandt (6) first drew attention to the root-like character of part of the rhizome of *Polytrichum*, which resembles a typical angiospermic root in possessing:

A. A piliferous layer of thin-walled cells bearing rhizoids. These latter, however, are thick-walled and are borne in such large numbers that they often form a dense tangled mass as thick as the rhizome itself; and in a large number of specimens examined the rhizoid mantle was so dense that it completely prevented the penetration of either dye or potassium nitrate to the piliferous layer of the rhizome. This, however, was not invariably the case, so that the piliferous layer has sometimes, apparently, an absorbing function.

B. An endodermis which, however, differs from that of any root in that it is discontinuous, being broken in three places by thick-walled cells which merge into thin-walled cells and form what Tansley and Chick have called 'The Radial Strand'.

C. A pericycle immediately inside the endodermis, thin-walled, and containing no starch.

D. Thick-walled, elongated cells, 'The Stereides', scattered amongst which are larger cells often united in bands of two or three, the cells of each band being separated by extremely delicate cellulose walls, which are comparatively devoid of pits. These cells Haberlandt has termed the 'Hydroids', and he has regarded them as conducting cells.

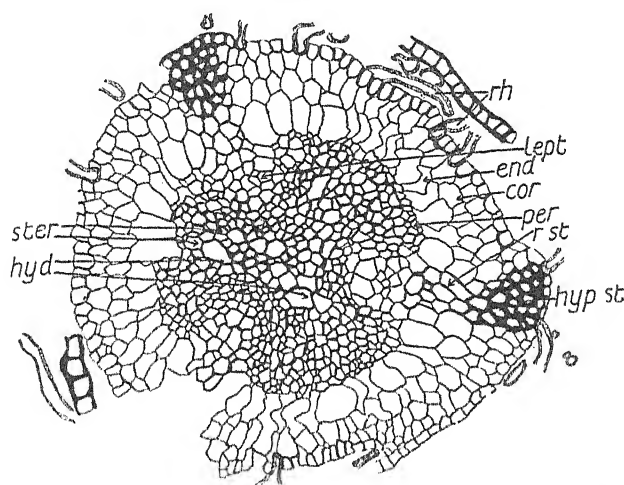
E. Cells resembling sieve-tubes, which have been found internally to the radial strand (Text-figs. 3 and 4).

The root-like region of the rhizome is extremely limited. The following table shows the ratio of this length of absorbing area to the length of the plant in a number of cases.

TABLE XIII.

Plant No.	Height of plant in cm.	Length of absorbing area in cm.	Ratio of length of absorbing area to height of plant.
1	24.6	1	0.04
2	17.5	1.14	0.065
3	14.8	1	0.07
4	18.1	1.9	0.1
5	22.8	1.2	0.05
6	17.8	0.5	0.03
7	4.7	0.5	0.11
8	8.9	0.5	0.06
9	22.8	0.5	0.022
10	3.8	0.5	0.134
11	23.4	1.5	0.064
12	24.6	1.6	0.067

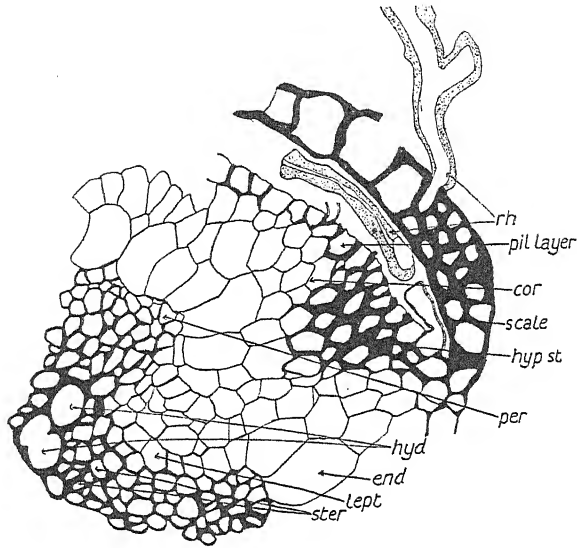
Immediately above the root-like, absorbing region of the rhizome the hypodermis becomes extremely thickened and the root-like characters are



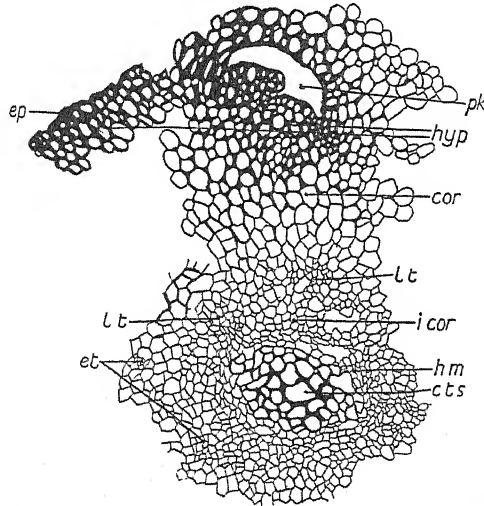
TEXT-FIG. 3. Transverse section of root-like region of the rhizome of *Polytrichum commune*. (*rh.* = rhizoids; *cor.* = cortex; *end.* = endodermis; *hyp. st.* = hypodermal strand; *r. st.* = radial strand; *lept.* = leptome, containing the cells resembling sieve-tubes; *ster.* = stereoides; *hyd.* = hydroids). $\times 240$.

lost. This transition from root to typical aerial stem has been described in detail by Tansley and Chick (8).

The aerial stem consists of a central cylinder of elongated cells, uniformly thickened, and possessing very few pits. Surrounding this cylinder is a layer of thin-walled, smaller cells which Tansley and Chick (8) have called the 'hydrom mantle'. This tissue merges externally into other thin-walled cells which form the rudimentary pericycle and which in turn is surrounded by the larger thin-walled cells of the cortex. The



TEXT-FIG. 4. Part of transverse section of rhizome of *Polytrichum commune*. (rh. = rhizoids arising from piliferous layer and scale; pil. layer = piliferous layer; hyp. st. = hypodermal strand; cor. = cortex; end. = endodermis; per. = pericycle; lept. = leptoids; hyd. = hydroids; ster. = stereoides). $\times 600$.



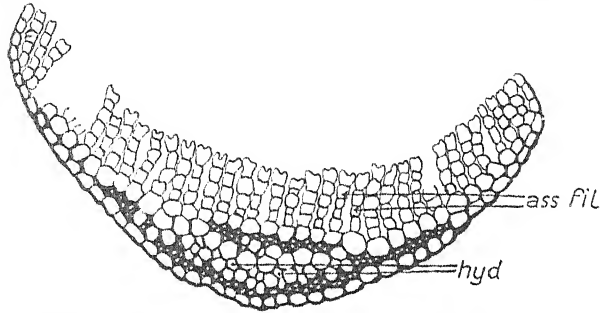
TEXT-FIG. 5. Part of transverse section of aerial stem of *Polytrichum commune*. (ep. = epidermis; hyp. = hypodermis; pk. = pocket enclosed between leaf-base and stem; cor. = cortex; l. t. = leaf-trace; h. m. = hydromantle; c. t. s. = central thickened strand; i. cor. = inner cortex). $\times 240$.

epidermis and hypodermis are composed of thick-walled cells, the hypodermis consisting of from three to seven layers of cells (Text-fig. 5).

All the tissue of the stem stele, with the exception of the central

thickened cylinder, arise from the leaf-traces. The leaf-trace in *Polytrichum* consists of three layers of thin-walled cells arranged parallel to the surface of the leaf. Two series of narrow empty cells, hydroids, lie between the three rows and alternate with them (Text-fig. 6).

The trace passes through the outer layers of the stem and reaches the smaller, thin-walled cells forming the extreme internal layer of the cortex



TEXT-FIG. 6. Transverse section of leaf of *Polytrichum commune*. (ass. fil. = assimilating filaments; hyd. = hydroids). $\times 240$.

or the rudimentary pericycle. Here the trace loses its band-shaped form and the cells increase in number. The hydroids from the various traces collect into a circular strand and join the thin-walled layer of cells forming the hydrom mantle.

Haberlandt (6) postulates a rapid conduction of water up the central thickened cells to the tip of the plant, so supplying the sex organs, or the sporophyte, as the case may be. At the same time he suggests that water passes out from the central strand into the 'hadrome mantle' and so up the leaf-traces into the leaves. The data already reported above, which demonstrate the efficiency of the external conducting system of *Polytrichum*, although showing that a sufficient supply of water can be obtained by the plant without internal conduction, do not rule out the possibility of the occurrence of such. It therefore seemed desirable to determine definitely whether internal conduction does occur in this plant and, if so, to what extent.

As a preliminary step experiments were carried out with a solution of eosin. External conduction was prevented by carefully shaving off all vestiges of leaf-bases for 1 cm. at the base or cut end of the plant. Plants so treated were then placed in stands in bowls of eosin solution. After varying intervals sections were cut and examined for the presence of stained tissue, the stain being an indication of the rise of the dye to that point in the stem. Eosin was found to be a very unsatisfactory dye, for the coloration obtained by its use differed but little from the normal yellow, and yellow-brown colour of the unstained tissue, and no sound conclusion could be drawn from its use. Trials were therefore made with 0.05 per cent.

solutions of methyl blue, methyl green, iodine green, and gentian violet. The last named proved to be the most effective, and plants treated as described above were therefore placed in stands in bowls of this stain. Sections were taken at intervals to determine the height to which the stain had ascended internally, and the results are shown in the table below :

TABLE XIV.

Plant No.	Height of plant in cm.	Time.	Height of rise of gentian violet in cm.	Ratio of height of rise to height of plant.
1	9	1 day	0.22	0.024
2	7	"	0.6	0.86
3	12	"	0.51	0.43
4	14	2 days	2.5	0.179
5	20	"	1.5	0.07
6	8	"	1.75	0.22
7	16	3 days	2.8	0.175
8	12.6	"	1.5	0.119
9	13	"	1.7	0.13
10	19	4 days	1.4	0.074
11	18	"	2.9	0.16
12	10	"	2.0	0.2
13	9.2	5 days	2.0	0.22
14	13.5	"	3.8	0.208
15	20	"	2.7	0.135
16	12	18 days	2.9	0.24
17	9	"	3.0	0.3
18	23	"	3.6	0.157

Other plants were treated as above and placed in stands in a 0.1 per cent. solution of potassium nitrate. After varying periods sections were cut at different levels on the stem and the presence of potassium nitrate was tested for by mounting in diphenylamine in concentrated sulphuric acid, the sections being immediately examined for the characteristic deep blue colour. The results obtained were as follows :

TABLE XV.

Plant No.	Height of plant in cm.	Time.	Height of rise of potassium nitrate in cm.	Ratio of height of rise to height of plant.
1	27.5	18 hrs.	1	0.04
2	10.5	1 day	0.38	0.36
3	12	"	0	0
4	13	"	1.2	0.09
5	15	3 days	3.5	0.23
6	10	5 days	2.5	0.25
7	12	"	3	0.25
8	20	"	4	0.2
9	15	18 days	5.5	0.37
10	12	"	7	0.58
11	18	"	13	0.72

Yet other plants similarly treated were placed in stands in bowls of 0.5 per cent. solutions of lithium sulphate. After varying intervals the plants were removed, cut into pieces 0.5 cm. long, and incinerated in a bunsen flame. The flame was examined spectroscopically for the lithium line, with the following results:

TABLE XVI.

Plant No.	Height of plant in cm.	Time.	Height of rise of lithium in cm.	Ratio of height of rise to height of plant.
1	11.5	10 mins.	2	0.174
2	21.5	18 hrs.	2.5	0.12
3	12.5	"	5.5	0.44
4	13.5	1 day	6	0.44
5	17	"	4	0.3
6	15.5	2 days	5	0.32
7	23	"	7	0.3
8	18.9	3 days	7	0.37
9	16	"	10	0.62
10	20.5	4 days	8	0.4
11	17	"	8.5	0.5
12	13	5 days	10.5	0.8
13	19	"	12	0.6
14	27	"	12.5	0.44
15	30	6 days	25	0.82
16	20	"	20	1
17	14	"	14	1

A comparison of the above three tables shows that the rate of absorption and internal conduction of lithium sulphate is the most rapid of the three liquids tested, the lithium reaching the tip of the plant in six days. Next in rapidity of penetration to the lithium sulphate solution is the solution of potassium nitrate, a salt readily absorbed by plants; while the rate of entry and conduction of gentian violet is extremely slow, probably on account of the absorption of the stain by the cell walls. In order to measure the rate at which solutions are absorbed by the rhizome, plants were placed in solutions of gentian violet and potassium nitrate, and, after definite intervals, sections were cut and examined for the presence of these substances.

The entry of gentian violet was extremely slow, coloration of the central strand being evident only after the plants had been left in a 0.05 per cent. solution for two or three days, by which time coloration of the cortex and hydrome sheath had taken place for a distance of 2 cm. or 3 cm. In some cases, at the end of a week the solution of dye had not entered the thickened cells of the rhizome.

A 0.1 per cent. solution of potassium nitrate was more readily absorbed, and complete coloration of the absorbing region of the rhizome, including the central strand, was apparent after 12-24 hours. Sections cut above the absorbing zone showed that in many cases internal conduction

occurred in the hydrom mantle and inner cortex rather than in the central thickened cells, and where a certain amount of conduction occurred in both these tissues the conduction was often more rapid in the hydrom mantle than in the central cells. The conclusion drawn from these observations was that the central thickened region of the rhizome is not a specialized conducting strand.

The three previous tables relate to experiments which were carried out with whole plants which had their leaf-bases removed, so that the liquid had to penetrate into the central strand and then rise in the latter.

Experiments were then carried out with plants whose rhizomes had been cut through, the cut ends of the stems being exposed to the liquid so that the rate of internal ascent, independent of penetration, was measured. External conduction in these plants was prevented by removing all tissue outside the central strand for a distance of 1 cm. from the cut end of the stem. These plants were then placed in stands in bowls of potassium nitrate, sections being taken at intervals and tested as before. The figures obtained are given in the following table:

TABLE XVII.

Plant No.	Height of plant in cm.	Time.	Height of rise of potassium nitrate.	Ratio of height of rise to height of plant.
1	12.2	1 hr.	1	0.08
2	16	5 hrs.	1	0.06
3	10	1 day	2.5	0.25
4	15	"	1.5	0.1
5	12	"	3	0.25
6	27	"	8	0.3
7	23	"	7	0.31
8	7.9	"	2.3	0.3
9	14	"	1	0.07
10	12.5	"	2.5	0.2
11	23	"	5	0.22
12	6	2 days	1	0.16
13	23	"	4	0.17
14	12	3 days	3	0.25
15	16	"	5	0.3

Plants similarly treated were placed in stands in bowls of lithium sulphate, cut into pieces, and incinerated at intervals as before, with the following results (see Table XVIII).

The more rapid rise is here again obtained with lithium sulphate, which takes about two days to rise to the tip of the plant when presented to the cut end, as compared with six days when complete plants were used. Even so, the rate is extremely slow when compared with the rate of external conduction.

An attempt was made to compare the rate of internal and external conduction in one and the same plant, and the only method possible was

by the use of gentian violet. The plants were placed in stands in bowls of this stain, left for varying periods, and the height of external rise noted. They were then sectioned at different levels, and the height of internal rise also noted. The results obtained are shown in the table below, although it must be clearly understood that the use of gentian violet as a criterion of the rate of internal conduction is not by any means satisfactory.

TABLE XVIII.

Plant No.	Height of plant in cm.	Time.	Height of rise of lithium.	Ratio of height of rise to height of plant.
1	9	10 mins.	3	0.33
2	7	"	1.5	0.21
3	11	30 mins.	2	0.18
4	10	1 hr.	2.5	0.25
5	12	3 hrs.	5	0.41
6	10	4 "	4.5	0.45
7	12	18 "	7	0.58
8	10	1 day	4	0.4
9	16	"	8	0.5
10	12.5	"	7.5	0.6
11	10	2 days	10	1
12	12	"	9	0.8

TABLE XIX.

Internal and external rise of gentian violet on whole plants of *P. commune*, the plants being submerged to the level of the dead brown leaves.

Plant No.	Time.	External rise in cm.	Internal rise in cm.	Ratio of internal rise to external rise.
1	1 day	6.3	0.5	0.08
2	"	7.8	0.5	0.064
3	"	1.7	1	0.06
4	2 days	8.9	0.7	0.078
5	3 days	9.3	2.4	0.25
6	"	5.1	2.4	0.5
7	"	4.8	1.5	0.3
8	4 days	7.5	2.2	0.3
9	"	8.7	2.1	0.24
10	"	8.3	1.9	0.23
11	5 days	8.3	3.8	0.45
12	"	9.8	6.3	0.64
13	18 days	10	8.2	0.83
14	"	9.4	7.3	0.77
15	21 days	15.2	6.6	0.44

It is evident from all the above data that, whereas an internal conduction of water does take place in *Polytrichum*, this method of ascent is of little importance when compared with the external system, for by means of the latter water is conducted to the tip of the plant at a relatively much more rapid rate.

MODE OF ENTRY OF WATER INTO THE PLANT.

It is clear that the externally conducted solutions are of no use to the plant unless they can be absorbed either by the leaves or by stem tissues. The stems of *P. commune* are furnished with a thick-walled epidermis and a several layered, thickened hypodermis which offers a formidable barrier to solutions conducted on the exterior. An attempt was therefore made to ascertain the path of entry of the externally conducted solutions into the stem.

Experiments were carried out with a 0.05 per cent. solution of gentian violet and with a 0.1 per cent. solution of potassium nitrate.

Two series of experiments with gentian violet were set up :

A. Complete *Polytrichum* plants were placed in dishes of water and in the axils of their upper leaves small particles of dye were carefully inserted. Sections of the upper regions of the stem taken after two days showed coloration of the cortex, this coloration being deepest in those areas associated with the leaf-bases, and extending from these areas down, and not up, the stem. Entry had therefore obviously occurred in the region of the apex.

B. Other complete plants were inverted in a solution of gentian violet with only their apices immersed in the liquid. Sections taken after two days showed dense coloration of the cortex, but no staining of the central thickened region occurred until six days had elapsed.

Previous experiments reported above show clearly that the entry of gentian violet is no true indication of the entry of water and salts, and so further experiments were carried out with potassium nitrate.

Complete *Polytrichum* plants were placed in a 0.1 per cent. solution of potassium nitrate. After definite intervals they were removed and sections were cut and tested for the presence of potassium nitrate by means of diphenylamine, the sections being examined as rapidly as possible after being placed in the reagent on account of the transient nature of the colour reaction. The following example is typical of the results obtained for plants left in the solution for one day.

Plant (A) had a total length of 19.8 cm. Sections after 24 hours' immersion of the basal region in potassium nitrate solution showed :

1. Complete coloration of all tissues from the tip to a level 2.3 cm. below the tip.
2. Coloration of the outer cortex, small portions of the inner cortex, and hydrom mantle in the regions of the leaf-traces, with no coloration of the central strand, extending over a distance of 14 cm. below 1.
3. No coloration at all for a distance of 1.5 cm. below 2.
4. Complete coloration down to the base—a distance of 2 cm. This means, clearly, that the potassium nitrate had entered the base and had

passed up for a distance of 2 cm. internally, but had not risen above that level. The salt had risen externally to the tip, had entered through the leaf-bases at the apex of the plant, penetrating the upper portion completely, and had passed down into the inner cortex and hydrom mantle through the leaf-traces.

Careful investigation of a number of complete plants which had been treated as described showed that the liquid had risen to the tip of the plant and that entry had taken place chiefly through the apex of the stem. In order to determine whether entry takes place in any other region than the apex of the stem, the apices were cut off and the wound blocked with either paraffin wax or collodion. Plants treated in this manner were put in stands into potassium nitrate solution and sections were taken at various levels. The following example is typical of the results obtained after 24 hours.

Total length of plant = 17.5 cm. Sections mounted in diphenylamine showed :

1. Complete coloration of all tissues from the tip to a level 2.5 cm. below the tip.
2. Coloration of the outer cortex, small patches of the inner cortex and hydrom sheath in the region of the leaf-traces, with no coloration of the central strand, for a distance of 3 cm. below 1.
3. No coloration for 12 cm. below, i. e. to the base of the plant.

In this experiment it is evident that no entry of potassium nitrate occurred at the base of the plant, but that an external conduction of solution took place, the solution entering the stem in the region of the green leaves near the tip of the plant.

In other experiments the whole of the portion of the stem bearing green leaves was removed and the upper and lower cut surfaces blocked with wax. The plants were treated as above and sectioned after 24 hours with the following typical results :

A plant 11.5 cm. long was sectioned, the sections being mounted in diphenylamine. An examination of these sections showed :

1. Complete coloration of all tissue from the tip to a level 2 cm. below the tip.
2. Coloration of the outer cortex, patches of the inner cortex and hydrom sheath in the regions of the leaf-traces, with no coloration of the central strand extending over a distance of 4.5 cm. below 1.
3. No coloration at all for a distance of 5 cm. below, i. e. to the base.

Entry, therefore, occurs all along the stem in the region bearing both the green leaves nearer the tip and the brown leaves found lower down on the stem, but it was evident that the greatest amount of water and salts entered at the upper end of the plant, whether that plant were whole or whether the tip had been removed.

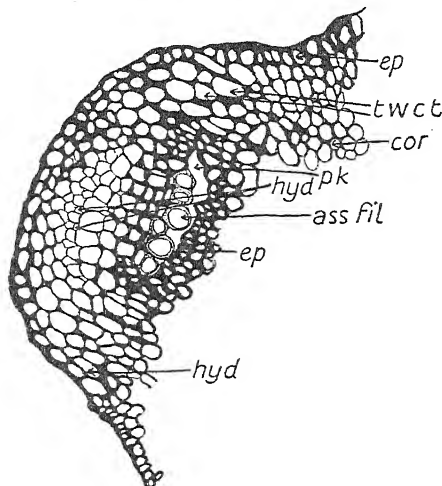
Careful examination of sections giving the colour reaction indicating

the presence of potassium nitrate showed that there were two paths by which the solution entered the stem:

A. Through the thin-walled tissue at the leaf-base.

B. Through the leaf-trace.

At the junction of the leaf-base with the stem, the thick-walled hypodermis is interspersed with thin-walled cells which place the thin-walled



TEXT-FIG. 7. Transverse section of leaf and part of stem of *Polytrichum commune*, showing thin-walled conjunctive tissue connecting the thin-walled tissue of stem and leaf. (*ep.* = epidermis; *hyp.* = hypodermis; *cor.* = cortex; *t.w.c.t.* = thin-walled connecting tissue; *hyd.* = hydroids; *pk.* = pocket enclosed between stem and leaf; *ass. fil.* = assimilating filaments extending down into pocket). $\times 240$.

tissues of the cortex and leaf-base in direct continuity (Text-fig. 7). It is along this path that the solution enters the outer cortex of the stem and gradually diffuses through the whole of the cortex. It is the quicker means of entry, for in all sections examined the first indication of colour reaction was seen in the region of the leaf-base in the outer cortex.

The experiments, previously recorded, in which salt was deposited at the tips of the plants, showed that this salt accumulated largely between the lamellae of the leaves, proving that external conduction had taken place even there. In experiments with potassium nitrate transverse sections of the leaves showed complete coloration of all tissues. It is clear, therefore, that the externally conducted liquid penetrates into all the leaf tissues. This coloration could be seen very clearly in the leaf-traces during their passage through the cortex, right into the hydrom mantle of the stem.

DISCUSSION AND CONCLUSIONS.

The results of the foregoing work show clearly that, contrary to pre-conceived ideas, the main water supply of *P. commune* passes up over the

external surface of the plant in the form of capillary films between the closely adherent leaf-bases and the stem; this upward passage being facilitated by the dense arrangement of the leaves and by the tufted habit of the plants. Under normal conditions, as a result of direct rainfall, dew deposit, and this external conduction, copious supplies of water reach the apex of the plant. This water is absorbed by the leaf tissues, diffusing into the peripheral layers of the stem in a lateral direction at the points of junction of the leaf-base and stem, and passing into the internal tissues of the stem in a downward direction through the leaf-traces. The central thickened strand in the stem of *P. commune* takes very little part, if any, in the upward conduction of water. In the light of the results of the present investigation this strand can best be regarded as a strengthening and supporting tissue which might also function secondarily as a store-house for water and reserve food materials. This suggestion would explain the presence of starch grains, oil and proteids found in the hydroids by Oltmanns (9) and Bastit (1), and would agree with their interpretation of the water-storing function of the central strand. This is quite contrary to the findings of Haberlandt (6), who demonstrated a considerable water-conducting capacity on the part of this tissue. The greater number of his experiments, however, were carried out with eosin solution, which the writer has found to be extremely unreliable owing to the similarity of the colour of the stain to that of the normal colour of the central stem tissue, with a consequent difficulty in determining at all correctly the limits of the unstained region. He also carried out a number of experiments with lithium sulphate, but, in many of these, any possibility of external conduction was ignored. Complete stems were used, and any rise of salt was regarded as being an internal rise. In some of his experiments, however, he removed the leaves from the base of the stem. In these he demonstrated a rapid rate of ascent of the solution, e.g. one plant whose total length was 6 cm. had conducted lithium sulphate to its tip in 5 minutes; another plant 7 cm. in length conducted lithium sulphate to height of 3.4 cm. in the same time. So rapid were his rates of ascent that he even compared the rate of conduction in *P. commune* with that in Angiosperms, which seems doubtful, on the face of it, in view of the uniformly thickened, sparsely-pitted cells of the central strand of *Polytrichum*, which cannot be expected to show the same rapidity of conduction as do the vessels of the higher plants. Such a rapid rate of conduction as Haberlandt claims to have taken place internally has never been attained at all in any of the writer's experiments.

ti Haberlandt suggested that water passing up the central strand would tldiffuse out into the surrounding hydrom mantle, in which the hydroids of the leaf-trace terminate, and that water would then be conducted upwards in the hydroids of the leaf-trace into the leaves. Further, he showed in his experiments that in many cases the conducting capacity of the hydrom

mantle was greater than that of the central tissue, for sections of plants placed in eosin solution revealed a staining in this tissue above the level of the stain in the central tissue. Similar results have been obtained by the writer, but in the light of the present work the conclusion is drawn that the stain had risen externally, passed into the leaf-traces, and then down through the leaf-trace into the hydrom mantle.

The water-supply passing up over the external surface of the plant would obviously tend to accumulate at the tip, as has been proved by experiments showing an accumulation of salt crystals at the tip of the plant when the bases were immersed in sodium chloride solution. This water would tend to maintain a condition of maximum turgidity in the rapidly growing cells at the apex, and therefore would ensure a maximum rate of growth. At the same time, surplus water, held in the form of films between the young leaves at the apex, would surround the sex organs and ensure a maximum supply for their development and for the process of fertilization, and would also be available for the rapid growth of the young developing sporophyte, supplying this structure directly through the archegonium neck. Haberlandt postulated a supply to the young sporophyte as a result of rapid passage through the thickened central strand of the gametophyte, a source which, in view of the lack of organic connexion between the sporophyte and this strand, seems much more improbable than the external supply suggested as a result of the present work.

If the Bryophyta stand at a fairly low level in the evolution of a land flora, they might be expected to retain many of the characteristics of their presumably algal ancestors. One of the most distinctive features of such plants is their power of absorbing water all over their external surface. The retention of this power by their Bryophytic descendants is a not unreasonable supposition, the higher forms of this group having developed a central thickened strand primarily for purposes of support in their sub-aerial habitat.

The writer wishes to take this opportunity of expressing her sincere thanks to Dr. F. A. Mockeridge, of University College of Swansea, for suggesting this work and for the valuable help she has given and the useful criticisms she has made during its progress.

SUMMARY.

1. The form and structure of the leaf-bases of *Polytrichum* and their mode of attachment to the stem are described.
2. The effect of heat and the humidity of the atmosphere on the angle of divergence of the leaves from the stem is determined.
3. The fact that water is conducted over the external surface is established. The rate of this conduction is determined by a number of methods, and the amount of water so conducted is calculated.

4. The entry into the stem of externally conducted liquids is observed to take place through the leaf-bases and leaf-traces, especially at the apex of the plant, and a lateral and downward passage of the liquid in the stem is shown to occur.

5. A description is given of attempts made to determine the extent of the conducting capacity of the central strand.

6. It is suggested that the function of the central strand is supporting and strengthening rather than conducting.

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EXPLANATION OF PLATE VII.

Illustrating Miss E. J. Bowen's paper on 'Water Conduction in *Polytrichum commune*'.

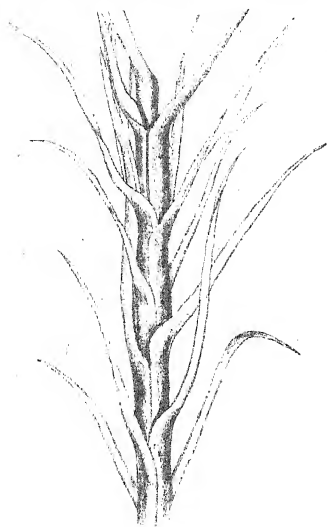
Fig. 1. Portion of *Polytrichum commune* plant, showing the normal angle of divergence of leaves. $\times 5$.

Fig. 2. Portion of plant in saturated atmosphere, showing reflexed leaves. $\times 5$.

Fig. 3. Portion of plant in wilted condition, showing leaves adpressed to stem. $\times 5$.

Fig. 4. Complete plants, with bases placed in 0.5 per cent. NaCl solution, showing salt deposit accumulated on stems and leaves after five days.

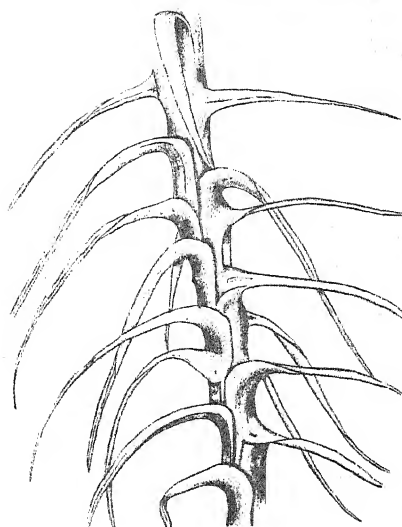
Fig. 5. *Polytrichum* plant, the cut stem of which, blocked with wax, was placed in 0.5 NaCl solution, showing salt deposit accumulated on stem and leaves after five days.



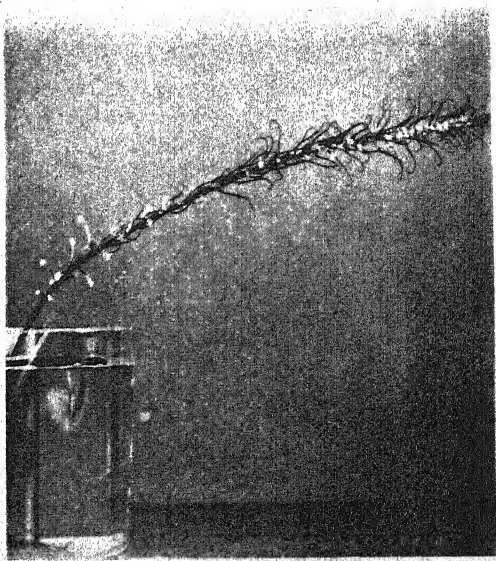
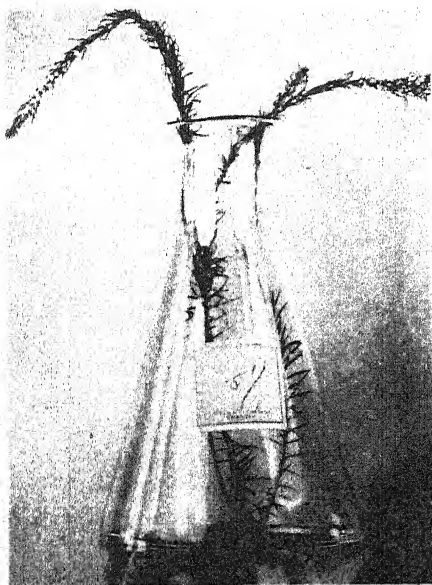
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The Structure of the Starch Layer in the Glossy Petal of *Ranunculus*.

BY

JOHN PARKIN, M.A., F.L.S.

With nine Figures in the Text.

INTRODUCTION.

IN a recent paper on the Glossy Petal of *Ranunculus* (2, p. 742) it was stated that the dense starch layer which lies immediately below the upper epidermis of the glossy part of the petal is apparently more than one cell thick. Only transverse sections of these petals had then been examined, and certainly from the appearance of the layer so viewed it looked as if it were two to three cells deep, as shown in Text-fig. 2 A of that paper (2, p. 741), and in Fig. 1 of this paper. The individual cells vary in size and are not regularly arranged in rows; but are, as it was then expressed, packed together somewhat after the style of crazy pavement.

Longitudinal microtome sections have since revealed the cause of this unusual appearance in transverse section. The starch layer is in fact only one cell thick, but owing to the starch-containing cells being arranged neither at right angles nor parallel, but *obliquely* to the surface of the petal, two or three tiers of these cells, instead of one, are cut in transverse section, thus giving the false appearance of a starch band two to three cells deep.

DEVELOPMENT AND STRUCTURAL FEATURES.

The development of the petal, with special attention to the starch layer, has been followed, chiefly by means of longitudinal sections in four common species of British Buttercups, viz., *Ranunculus Ficaria* L., *R. acris* L., *R. bulbosus* L., and *R. repens* L. This is essentially the same in each except in one surprising difference, not at first obvious, which is described in a later paragraph.

The following details given of the development refer to the petal of *Ranunculus Ficaria*:

Stage 1. In the earliest stage examined the petal had attained a length only of 0.8 mm. A mature petal may be assumed as having a length of 10 to 12 mm. The outline of this young petal, as viewed in

longitudinal section and magnified $\times 50$ is given in Fig. 2. It is interesting to note that at this early stage the nectary depression *n* and its ventral outgrowth *ns*, destined to become the nectary scale, are quite in evidence. In Fig. 3 the upper part of this petal, taken about the region marked with a cross in Fig. 2, is shown in detail magnified $\times 150$. (For the sake of clearness in this and the five following figures of the same magnification only the cellular contents, viz. nuclei and starch granules of the upper epidermis and starch layer, are shown. For the same reason vascular elements are omitted.) The cells throughout at this early stage are very similar, and are arranged more or less in six layers, two epidermal and four mesophyll. Lower down, where the petal is a little thicker, there may be five mesophyll layers. The uppermost of these, the one immediately below the upper or ventral epidermis, is destined to become the starch layer, *st*. At this stage it shows hardly any differentiation.

Stage 2 (Fig. 4). The petal has lengthened to 3 mm. The ordinary mesophyll cells have begun to elongate in the usual, i.e. the longitudinal, direction. On the other hand, the cells of the starch layer have lengthened somewhat in the opposite direction, viz., at right angles to the surface of the petal. Starch granules have not yet begun to be deposited in them.

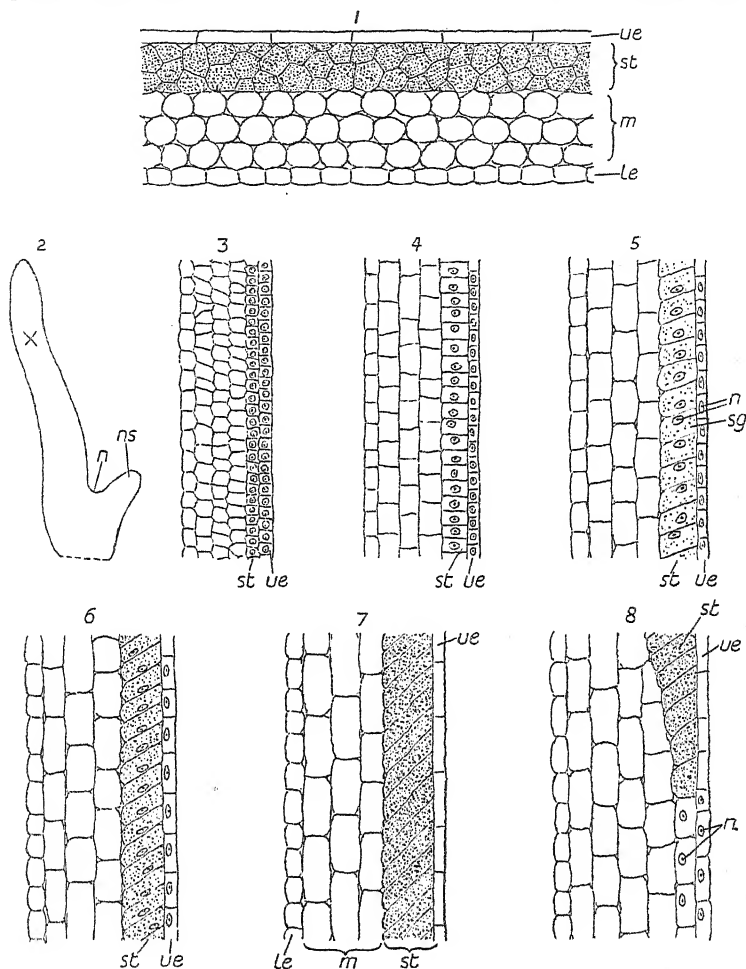
Stage 3 (Fig. 5). The petal is now 5 to 6 mm. long. The cells of the starch layer have commenced to take an oblique direction, and granules of starch, *s.g.*, are now visible in them.

Stage 4 (Fig. 6). Length of petal 7 to 8 mm. A stage shortly before maturity. The oblique character of the starch cells and the starch deposit in them are more apparent. Nuclei are still evident in these cells as well as in the upper epidermis. Though the petal is now deeply yellow it is not yet glossy.

Mature Stage (Fig. 7). The obliquity of the starch cells is considerably accentuated, and the nuclei have disappeared from them. Each cell now consists apparently of a mass of minute starch grains surrounded by a delicate wall of cellulose. The nuclei also, as well as the chromoplasts, have vanished from the upper epidermal cells, leaving them quite hyaline. The petal in consequence assumes its characteristic gloss.

Transition between Gloss and Mat Regions. As pointed out in my former paper (2, p. 740), and by other investigators, the basal parts of these glossy petals are always free both of starch and gloss. In Fig. 8 the transition between the glossy and mat regions in the mature petal of *Ranunculus Ficaria* is shown in longitudinal section. The oblique character of the starch cells gradually becomes less apparent and finally disappears as they become starchless. Nuclei and a few chromoplasts are discernible in both the upper epidermal and sub-epidermal cells of the mat basal region, though absent in the corresponding cells of the glossy part.

Apparently this peculiar oblique structure of the starch layer in the glossy petal of *Ranunculus* has not been made known before. The only

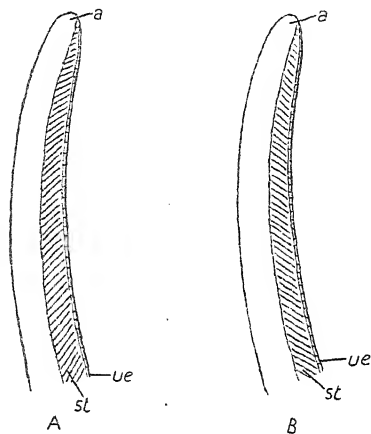


FIGS. 1-8 (semi-diagrammatic), showing the development and structure of the petal of *Ranunculus Ficaria* L. 1. Transverse section of mature petal through the glossy part. Other figures are in longitudinal section. $\times 150$. 2. Outline of the youngest stage examined: *n.*, nectary depression; *ns.*, outgrowths which become the scale of the nectary. $\times 50$. 3. Part of Fig. 2 (stage (1) of text) taken from the region marked by the cross. $\times 150$. 4. Stage (2) of text. $\times 150$. 5. Stage (3) of text. $\times 150$. 6. Stage (4) of text. $\times 150$. 7. Mature stage. $\times 150$. 8. Shows transition from upper glossy area to mat basal region. $\times 150$. *ue.*, upper epidermis; *le.*, lower epidermis; *st.*, starch layer; *m.*, mesophyll. Cell contents (nuclei, *n.*, and starch granules, *sg.*) are only indicated for the starch layer and upper epidermis.

drawing of a section of the mature petal of which I am cognisant is a Text-fig. in Köstlin's paper (l. p. 327). In this the starch layer is shown as a regular row of roundish cells below the upper epidermis. It might be guessed as such from a section cut by hand, and that was my impression more or less before using the microtome. The cell walls are very delicate,

and the abundance of starch granules prevent their being seen at all clearly. Their appearance as brought out in transverse and longitudinal sections was entirely unanticipated.

Finding that the longitudinal sections of the petals of *Ranunculus acris*, *R. bulbosus*, and *R. repens* revealed likewise oblique starch cells, one



FIGS. 9 A and 9 B. Diagram (not drawn to scale) of the upper part of the petal in longitudinal section to show the difference in the slope of the starch cells. 9 A. Direction taken by the cells in *Ranunculus Ficaria*. 9 B. Opposite direction taken by the cells in *R. acris*, *R. bulbosus*, and *R. repens*. (a., apex of petal; st., starch cells; ue., upper epidermis.)

naturally jumped to the conclusion that the structure of the starch layer would be essentially the same in all species of glossy *Ranunculus*; but doubt was cast upon such a generalization by the following observation.

After making the drawing of the longitudinal section of the mature petal of *Ranunculus Ficaria*, a similar section of one of the other species was being examined when it was noticed that the direction of the slope of the starch cells was the reverse of that represented in the drawing. On referring to the section of *R. Ficaria* it was found that no error of delineation had been committed as one at first imagined; and the unexpected and interesting detail came out that the starch cells in the petals of *R. acris*, *R. bulbosus*, and *R. repens* are sloped in the opposite direction to that in *R. Ficaria*. The diagram (Fig. 9) will make this distinction clear. In *R. Ficaria* the slope of the starch cells is directed downwards, i.e. towards the base of the petal and inwards, i.e. towards its interior. In the other three species the slope is just the opposite, viz., upwards, i.e. towards the apex of the petal and inwards.

COMMENTS.

Since this difference in the obliquity of the starch cells occurs specifically where it might be expected on general grounds of affinity, there is little doubt but that these three common Buttercups, *R. acris*,

R. bulbosus, and *R. repens*, are more closely related to each other than they are individually to *R. Ficaria*—a species which stands considerably apart within the genus.

In view of these two opposite types of obliquity in the starch cells it is difficult to imagine the one changing by a process of reversibility into the other. It seems easier rather to conjecture that from an original layer of starch cells with no obliquity two lines of evolution arose with opposite types of slope. The investigation is being continued with this possibility in view. Already indications of no slope in the starch cells has been observed in some species, notably in a New Zealand one, where primitiveness might be expected. In fact, a preliminary examination of several species of *Ranunculus* suggests that there may be considerable variation in the structure of the starch layer.

It is hoped, therefore, that the structure of this layer may have value in the working out of a phylogenetic scheme for the genus. At any rate the view already put forward (2, p. 752), that the glossy character of the petal may be of considerable importance in the natural classification of the genus, is strengthened by the discovery of the peculiar structure of the starch layer in these four species.

ACKNOWLEDGEMENTS.

Through the kindness of Professor J. H. Priestley the microtome work in connexion with this paper has been carried out in the Botanical Department of Leeds University by his laboratory assistant, Mr. A. Millard. To both I wish here to tender my grateful thanks.

SUMMARY.

1. The cells composing the starch layer in the glossy petal of the four species of *Ranunculus* investigated take an unexpected *oblique* course, thus presenting in transverse section the appearance of a layer two to three cells deep. In reality it is only one cell thick.

2. The development of the starch layer in the petal of *Ranunculus Ficaria* has been followed out in detail.

3. The direction of the slope of the starch cells in *R. acris*, *R. bulbosus*, and *R. repens* runs in the opposite direction to that in *R. Ficaria*. It is probable, therefore, that there may be considerable variety in the structure of the starch layer in the genus.

4. It is suggested that the structure of the starch layer may be of value in working out a phylogenetic scheme for the genus.

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NOTE.

NOTE ON SOME PLANT REMAINS FROM AN OLD CAUSEWAY IN ESSEX.—The following is a brief description of plant remains from the site of an old river valley near the north bank of the Thames estuary, at Southchurch, Essex. They were obtained by Mr. A. G. Francis, F.S.A., from a causeway constructed apparently between 800 and 500 B.C., the age being suggested by West Alpine remains and by associated fragments of Hallstatt-La Tène I pottery. The causeway, which is covered by 2 feet of estuarine clay and a varying thickness of surface soil, rests upon lacustrine clay overlying gravel, and is considered to have led from a landing-stage on the shore of a mere to a 'crannog', or lake-dwelling village, situated in the mere. Unfortunately, the projected building of bungalows has prevented excavations being carried sufficiently far to reveal the constructional details of the crannog itself, and what would, no doubt, be valuable botanical material is thus not available for examination. A full account, from the archaeological and geological standpoints, of the discovery, position, and construction of the causeway will be given elsewhere by Mr. Francis; it is sufficient here to make certain observations of interest botanically.

The causeway showed three types of construction :

- (1) at the landing-stage on what was apparently the shore of the old mere ;
- (2) where it crossed the floor of the mere ; and
- (3) where it passed over an islet in the mere.

(1) The landing-stage showed an uppermost layer consisting of two large trunks embedded in black mud and splayed towards the shore. One of these, 8 inches in diameter, was longitudinally split ; the other, 12 inches in diameter, was complete. Both retained their bark, and were identified as oak.

(2) The causeway over the floor of the mere formed a 'corduroy road'. (See accompanying diagram, which shows a representative section of the corduroy road.) It revealed a top layer of horizontal oak saplings from 4 to 5 inches in diameter ; these were in general placed 16 inches apart, parallel with the length of the causeway, and were embedded in and overlain by black mud.

In both these cases the larger timbers rested upon a 'fascine' work, the members of which were arranged in a parallel manner transversely to the long axis of the causeway. The fascine work consisted of the following layers :

(a) A 'wattle' layer composed of trimmed branches. These branches varied in diameter from about $2\frac{1}{2}$ inches downwards ; they were cut transversely or obliquely, and in some cases possessed thickened ends, indicating the point of junction with the parent stem. There were from one to five series of wattles, the members of each series being separated horizontally by 12 inches of soil, and the whole wattle layer averaging from 6 to 8 inches in depth. Its constituents were hawthorn, alder, and willow branches, for their structure was found to correspond

to that of *Crataegus monogyna*, *Alnus glutinosa*, and *Salix*; specific reference was not possible in this last case. In all the specimens sent for identification, the bark and wood were very soft and crumbling, and in consequence difficult to section; but small portions, both of transverse and longitudinal sections, occasionally revealed the structure comparatively clearly. The greater number of the 'wattles' examined were willow; alder was rare.

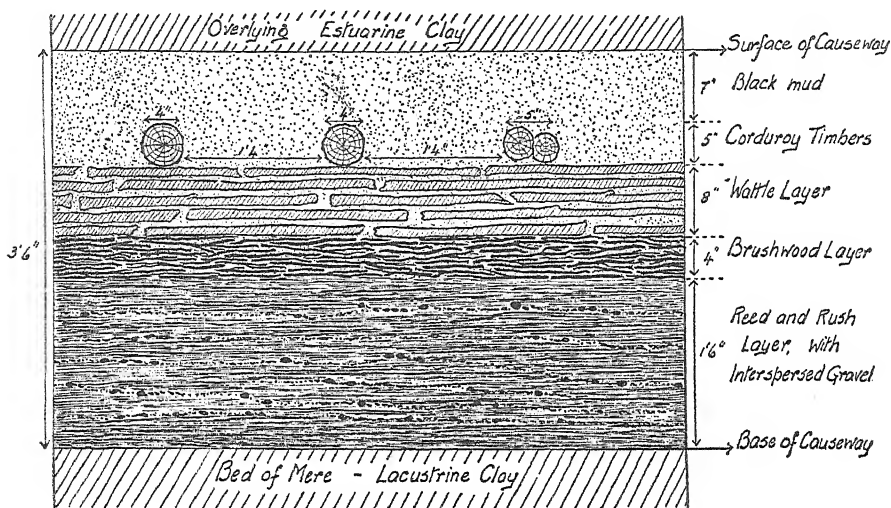


Diagram of part of a representative section of the 'corduroy road', taken transversely to its long axis, to show the general disposition of the plant material. (Scale of 1 inch to 1 foot.)

(b) A brushwood layer. This was composed of closely-arranged fine twigs retaining their bark; the layer varied in thickness up to 4 inches. These twigs disintegrated on handling, so that identification was generally impossible; their appearance *in situ* suggested, however, that they were of the same types as the wattles. The material composing the brushwood layer was macerated, sieved out, and carefully hand-picked; and a twig of the common elder (*Sambucus nigra*), showing the structure of the wood and pith very perfectly, was obtained, together with numerous elder and blackberry 'seeds', hawthorn fruits, and bramble prickles.

(c) A 'reed and rush' layer, forming the foundation of the causeway. This is so called because, as it lies *in situ*, it gives the general appearance of being composed of tied bundles of flat reed-like leaves and rush-like stems. These bundles were closely arranged; and layers of gravel alternated with layers of bundles, having been placed there, no doubt, for the purpose of keeping the plant material in place. The reed and rush layer varied in thickness, reaching as much as 1 foot 9 inches in some parts of the corduroy road. Its constituents were much decayed, and exact identification was impossible; but there were present what appeared to be the rook-stocks and adventitious roots of some such plant as *Juncus*, together with the remains of some rather thick aerial stems. Aseptate hyphae and a few hard-walled spore-cases of an indeterminate fungus also occurred in this decayed material.

(3) Where the causeway crossed the islet it was, of course, entirely sub-aerial and not partly submerged as in the other two cases. The corduroy timbers were absent; the fascine work was, however, of the same general construction as at the landing-stage and in the corduroy road, though not so deep, the reed and rush layer being only a few inches thick. From this layer of the islet road were obtained, on maceration, a few seeds (without embryos) having a shape and marking similar to those of the champions, and two small capsule-like structures. Each of the latter, when cut across, showed two compartments separated by a very thin partition; in one of the compartments of one capsule was a fairly large, but withered, seed. The outer layers of both capsules had disintegrated, revealing the course of the vascular strands; this, together with the shape and general structure of the fruits, indicated that they belonged to a Cruciferous plant, possibly of the type of scurvy grass (*Cochlearia officinalis*). At a bend of the road on the islet, a variation was shown in the arrangement of the wattles. Instead of being *all* parallel with the long axis of the causeway, members of alternate layers were arranged transversely to the axis, doubtless to give extra strength at this point.

From an ecological standpoint, the association of plant remains—oak, willow, alder, hawthorn, elder, bramble, with evidences of *Juncus*, champions and scurvy grass—in this ancient causeway is interesting. The builders of the Southchurch area in the last millenium B.C., it may be supposed, drew upon material near at hand and easily collected and transported. Oak forests originally occupied the London Clay and Brickearth soils of this area; and Brickearth formed the surface soil in the upper part of the old stream which fed the mere in which the causeway was built. It seems very probable, therefore, that the larger timbers—oak—were obtained from forest land carried by this Brickearth, and floated down the stream to the building site in the mere.

The wattles and brushwood—willow, hawthorn, alder, and elder—were no doubt cut from the margin of the stream, where the oak forest presumably passed into an 'alder-willow' association. (See Tansley, 'Types of British Vegetation', pp. 205, 206; also pp. 76–82.) The remains of brambles—prickles and 'seeds'—in the brushwood layer probably came from this association also. If, as appears likely, the brushwood layer was composed largely of the trimmings from the material which was to form the wattle layer, fruits of elderberry and hawthorn may have been still attached to the finer twigs. The presence of the seeds and fruits of elder, blackberry, and hawthorn in the brushwood layer suggests, as Mr. Francis has pointed out, that the foundation of the causeway was laid in the late summer when the water of the mere was at its lowest before the autumn and winter rains began.

The constituents of the reed and rush layer were probably gathered from the margin of the mere into which the causeway led.

Agriculture and building operations have now taken possession of much of the Southchurch area; an examination of the present-day flora of the neighbourhood, however, revealed the remains of an association of plant types such as that indicated above. On the London Clay are still some oak (*Quercus Robur*) woods, isolated portions of the once-extensive 'Royal Forests'. The Brickearth has been cleared of forest and now provides a rich corn-growing area, with elm (*Ulmus campestris*) as

the chief planted tree in the hedges and along the road sides. The margins of the streams, however, still possess willows in short stretches, with hawthorns and occasional elders and brambles. The alder seems to have disappeared from this immediate neighbourhood; it will be remembered that it was rare amongst the material examined from the causeway. The plants of the marsh land are typical of such a habitat, being chiefly species of Cyperaceae and of *Juncus*, with various tall grasses;ampions, meadowsweet, and other water-loving plants occur with the grasses along the stream sides and ditches, forming possibly a less halophilous association now than formerly, owing to the construction of dykes to prevent inroads of the sea.

The grateful thanks of the writer of this note are due to Mr. Francis for so kindly obtaining and sending the plant remains described, and for much useful information as to their occurrence, &c.; to Miss Jean Dickson for preparing the material for microscopic examination; and to Mrs. Clement Reid for very kindly identifying the elderberry seeds.

H. BANCROFT.

THE SCHOOL OF RURAL ECONOMY,
UNIVERSITY OF OXFORD.
September, 1930.

Studies in the Cytology of the Hibisceae.

III. A Study of the Prophase of the Nucleus of the Pollen Mother-cell of *Thespesia populnea*.

BY

W. YOUNGMAN.

With Plate VIII.

TECHNIQUE.

THE developing flower buds were taken from the tree and each as plucked, whilst the gatherer was still beneath the tree, had its covering of floral envelopes removed by cutting a ring around the bud with a sharp knife and lifting off the cap of calyx and corolla. The bud was then immediately thrown into a tube containing the fixing fluid. This method was found by experience to give the best results. Removal of the anthers from the buds before immersion in the fixing fluid was also tried. All the organs of a flower-bud contain so much mucilage, which on contact with the fixing fluid impedes penetration, that it was found best to damage the buds as little as possible. The mucilage, characteristic of the Malvaceae generally, is an impediment in the study of their cytology. Not only does it offer an obstacle to rapid penetration of the organs by many fluids, but also on contact with water it causes sections to come off the slide. Towards the end of the prophase, at the approach of heterotypic division in the microspore mother-cell, a mucilaginous pellicle forms around the cytoplasm. Many fixing fluids cannot penetrate this envelope sufficiently quickly to yield stages in the dividing nuclei. Flemming's Fluid suffers in this respect. Very fine fixation of the nuclear structures at early prophase has been obtained, however, with the use of Flemming's Stronger Solution (1 per cent. chromic acid solution, 45 c.c., 2 per cent. osmic acid solution, 12 c.c. and glacial acetic acid, 3 c.c.). The material was immersed in this solution for twenty-four hours and then washed in running water for fifteen hours. Washing for twenty-four hours, as so often recommended, was found to cause to some extent maceration of the cell contents, and so was reduced to a shorter period. The material was then taken up through alcohol by 10 per cent. increasing strengths. Bouin's Fluid (formalin 25 c.c., picric

Acid, saturated solution in water 75 c.c., glacial acetic acid 5 c.c.) was used with some success. In the search for a rapidly penetrating fixing fluid many solutions have been tried. None of them have proved ideal. Schaudin's corrosive sublimate solution (saturated watery solution of corrosive sublimate 95 parts, glacial acetic acid 5 parts, 90 per cent. alcohol 50 parts) was found to be rapidly penetrating and the most certain for late prophase and spindle stages, but the fixation with it is not of the very best. Very careful washing in water and treatment afterwards with iodine dissolved in 70 per cent. alcohol, when that stage is reached, are necessary to avoid the deposition of crystals when using this method. The rapidity of its fixation is due to the rather high percentage of acetic acid and ethyl alcohol that it contains. It has proved much superior to Carnoy's Fluid which, as a rapidly penetrating solution, has also been tried. The material after passing through alcohol and xylol was embedded in paraffin wax.

EARLY PROPHASE.

The early prophase of the nucleus of the pollen mother-cell shows its periphery to be traversed in various directions by thin chromatic strands. These strands when viewed under high magnification (Pl. VIII, Fig. 1) are seen to curve about the nuclear surface with many dot-like chromatic granules at intervals along them, and at places much larger irregular chromatic deposits. A nucleolus is generally present, situated near the periphery at one point, and from this nucleolus the thin spireme strands radiate. Whether the nucleolus be seen in a surface view of the cell or not will entirely depend upon which hemisphere be looked at. The spireme threads often cross one another, sometimes several doing so at one spot, and at such spots occur common aggregations of chromatic substance forming the so-called 'net-knots'. At this stage the threads appear to be single linear strands, no signs of doubleness being visible. They are exceedingly fine. At the next stage the threads appear more regular and less twisted along their course, although sweeping curves and hairpin-like bends in them are abundant (Pl. VIII, Fig. 2). The chromatin now is regularly distributed along the threads. The thread at this stage usually shows as a thin continuous, but curved, line of chromatic material, with granules rarely to be seen along it as they previously were. Next, distinct longitudinal doubleness in some of the threads (Pl. VIII, Figs. 3 and 4) is visible, and amongst the threads that do not show doubleness some are thinner than others. The thicker threads can sometimes be seen divaricating into two at places, whilst perhaps no sign of doubleness can be made out elsewhere along their length. Thus, even when they do not show this suggestion of doubleness, it seems legitimate to assume that they are so, consisting of two thin threads longitudinally fused.

This leads us on to very controversial ground. Are the two longitudinally fusing threads the two descendant portions of the spireme, derived in one case from the male gamete and in the other from the female, that fused at the time of fertilization of the ovule from which developed the plant that bears the pollen mother-cells now under consideration? Or are these fusing threads the reassociating halves of longitudinally split spireme lengths that separated at the telophase of the generation of cells from which the microspore mother-cells have come? It has not been found possible to give an answer to these questions from the material under consideration. The developmental phenomenon of the nuclear thread in passing from the generation previous to the spore mother-cell to that of the spore mother-cell itself wants to be studied, and for such observations one would not ordinarily choose so small a nucleus as that of *Thespesia* as ideal material for the elucidation of the problem. In addition to the fusing chromatic threads in the nucleus at this stage there is still a prominent nucleolus. The chromatic threads show as much more delicate line-like arrangements when stained with Heidenhain's iron-alum than when stained with acid fuchsin ('Fuchsin S'). It is possible that this difference is due to a central core of chromatic material being within the spireme, and that with iron-alum the central core only retains the stain whilst with the Fuchsin the whole spireme substance stains.

SYNIZESIS.

At first the single and pairing threads occupy the periphery of the nucleus, and at this stage the nucleus is at the centre of the cell. When pairing has generally taken place the thread contracts away from the periphery and comes into the inside of the nuclear cavity. The longitudinally-double thread balls up at one side of the nucleus. The nucleus thus comes to show clear regions unoccupied by the thread (Pl.VIII, Fig. 5). Sometimes the nucleolus lies to one side of the skein of thread, but there always seem to be some threads connected with it. Where these threads come off there is usually to be seen a globule-like projection of the nucleolus. This was noticed by Latter (12) and called by her the 'nucleolar body'. At least two threads join the nucleolus at this point. At other times the nucleolus may appear as if enveloped within the skein. The nuclear sphere now migrates through the cytoplasm and comes to lie at the periphery of the cell. A very delicate membrane limits the nuclear cavity internally at this stage.

From this point onwards a great difficulty is often encountered in deciding the sequence of events. The determination whether a particular stage follows or precedes another is often no easy matter, and anything that can be used as a guide is of importance. There are a few such helps.

Usually all the pollen mother-cells in the same anther loculus are approximately at the same stage of development at the same time. This synchronization of events may not be constant in all plants, for Digby (6) records the reverse in *Galtonia* with stages other than 'synapsis'. Prior to synizesis, which term is applied to indicate the stage of contraction and balling up of the spireme, the nucleus lies near the periphery at one place. One must be very perspicuous in deciding this last point, for a nucleus may be quite in the middle of a section of a cell but yet touch the periphery of the cell itself. By the strepsinema stage the pollen mother-cell will have become quite rounded in shape and lie separate from its neighbours in the anther cavity. From this last stage onwards a denser area of cytoplasm develops immediately around the nucleus. This is the perinuclear zone, and at about the same time a hyaline layer around and outside the cytoplasm begins to increase in thickness.

At synizesis, then, the double thread lies balled up at one side of the nucleus, which latter has migrated from the centre to the periphery of its cell. At first, even in the balled-up condition, it is possible to see signs of doubleness in the thread. Very soon, however, the mass becomes more compact, and the individual threads can no longer be traced.

From out of this compact mass hoops of thick, darkly chromatic spireme next emerge. At first the hoops have a horse-shoe shape with their apices all directed toward the pole away from the dark chromatic mass in which they are embedded (Pl. VIII, Fig. 6). Within this chromatic mass are the nucleolus, staining black like the hoops with Heidenhain's iron-alum, and the 'reserve' thread from which apparently the hoops expand outward. Pl. VIII, Fig. 6, is of a case where all the thread is seen in one section, and some seven loops can here be counted. The hoops emerge further from the mass, and as they do so they become less regular in their curvings (Pl. VIII, Fig. 7). The spireme has now opened somewhat from the former mass.

At this stage the spireme is thicker than it has appeared previously, and under high magnification shows a distinctly beaded appearance (Pl. VIII, Fig. 8). The beads show signs of right and left halves, the thick thread thus being longitudinally double. The hoops, though not so obviously horse-shoe-shaped as at first, are still widely open, and when seen in side view barely reach the periphery of the nuclear sphere toward the pole away from their origin. Seen from above or below they form a rosette of loops radiating from a centre (Pl. VIII, Fig. 9). This is regarded as the pachytene bouquet stage. An extreme difficulty with regard to the events now being described has been experienced in deciding the consecutive order of various stages. There is little or nothing to guide one in the matter at this phase. The stage shown in Pl. VIII, Fig. 7, for instance, resembles somewhat the condition shown by Digby (6) in her Figure 52,

which she describes as 'Beginning of second contraction'. If this be the interpretation then Pl. VIII, Fig. 6, might perhaps be regarded as a later and not a previous stage, representing the climax of second contraction. The stage in Pl. VIII, Fig. 6, has been antedated to that in Pl. VIII, Fig. 7, in part by the fact that the phenomenon of cytomixis, presently to be described, has not appeared in the first case, whilst it is present in the second. In a stage believed to follow very closely, if not directly, upon that just described, eight looped projections from a basal mass are to be seen in a side view (Pl. VIII, Fig. 11). These projections are of a different nature from the hooped forms previously seen. They consist of two threads arising close together, side by side, and twisting around one another, sometimes being so closely entwined as to resemble a single strand. These twisted strands are longer than were the horse-shoe-shaped hoops. This stage in side view is not at all easy to find, although more commonly seen in a view from above or below (Pl. VIII, Figs. 10 and 12). It is a matter of considerable odds against a section so cutting a cell as to show it in side view in a nucleus. To exhibit it the thinnest possible section is required that just shows an entire nucleus. That is to say, the knife wants to pass at one cut at a tangent to one end of a diameter of the nucleus, and at the next cut at a tangent to the other end; furthermore, the diameter has to be just the correct one to exhibit the loops. It will be understood that the element of chance is a very great one. Amongst the eight projections, as seen at this stage, two can sometimes be seen coming off close together, and these are shorter than the rest and seem to retain the horse-shoe-shape somewhat (Pl. VIII, Fig. 11). Two such loops are seen in a view from above in Pl. VIII, Fig. 12. Very often the nucleolus is encircled within another loop (Pl. VIII, Fig. 11). It is not possible to say if this be constantly the same loop. Probably this loop usually, or at all events sometimes, grows into a longer loop. There is reason to believe, as we shall see presently, that three loops remain shorter than the other five. Of these three the horse-shoe-like pair at this stage are probably two. The difficulty in identifying them is that the loops are growing or increasing in length in some way, perhaps by a pulling of more thread from out of the 'reserve' near the nucleolus; and in order to get a stage where we can see them all in one view we must have one before they have increased beyond the circular outline of the nucleus we are viewing. This confines us to rather narrow limits for deciding differences of length.

The sides of each of the loops soon appear as if they had come together proximally, and eventually they twist up into a cord-like strand. Distally they also twist, but for some time the twists here are very open ones. The nucleus, as already mentioned, just before the stage we are considering, migrated to the periphery of the cell. This movement of the nucleus may be toward the surface of its cell, where that surface adjoins

a neighbouring pollen mother-cell, or the movement may be toward the outside of the cell, where there is no neighbouring microspore mother-cell. At this stage the cytoplasm would not seem to fill the space within the cell walls. This appearance may be due to shrinkage in preparation, but in otherwise well-fixed material this condition is general, which rather suggests it as natural. The migration of the nucleus toward a neighbouring cell, when such a migration occurs in an anther loculus, would seem to be a general condition for all the cells of that loculus. Gates (9) comments upon the same fact in the case of *Oenothera gigas*.

Always at this synizesis stage the phenomenon of extrusion of chromatin from the nucleus takes place. Ejections into the cytoplasm of comparatively slight quantities of chromatin from the nucleus at the meta-phase stage in somatic divisions have been seen frequently to occur, but they would not seem to be so constant and consistent a phenomenon at that stage as is the process about to be described in the pollen mother-cells. This extrusion of chromatin from the nucleus at this stage has been observed in many flowering plants by various workers (Digby in *Galtonia* (3), in *Primula* (4), and *Crepis* (5), West and Lechmere in *Lilium* (17), Gates in *Oenothera* (9), and *Lactuca* (10), to mention but a few). Farmer and Digby (7) record chromatin expulsion in ferns, whilst phenomena suggestive of the same process are also known amongst the fungi.

Observations have been made in the course of the present work that indicate that the cells from which this chromatin expulsion has occurred continue their existence and divide at heterotypic division in the usual way. Gates (8) says: 'A very important point is whether nuclei in which extrusion has occurred afterwards complete the meiotic processes'. In *Thespesia populnea* it seems they do. The phenomenon has been found and would appear to be also a general one in the nearly related genus *Gossypium* and in the allied genus *Bombax*.

The phenomenon occurs at the stage when the spireme thread begins to open from the knot at synizesis. At this phase there is apparently a portion of the chromatic thread that does not enter into the formation of the loops. This thread passes out of the nuclear cavity into small teat-like projections of cytoplasm which pout out from the general cell-mass through pits in the party-wall and join the cytoplasm of a neighbouring cell. Along these cytoplasmic connexions some of the chromatic thread is voided into the neighbouring cell, where a clear vacuole-like area forms around it. Sometimes much more than the chromatin from some of the spireme thread passes out of the cell in this way. The entire nucleolus even may pass into the neighbouring cell. Evidences of this expulsion of chromatin may show later than the synizesis stage, although that would seem to be the usual period of its actual occurrence.

To return to the eight twisted threads projecting from the region of

the nucleolus and growing outward around the periphery of the nucleus towards the opposite pole. Proximally the part of each that is fixed in the reserve mass of thread consists of two strands twisted around one another, and viewed from the nucleolar pole they radiate out at this stage as eight arms from the region of the nucleolus as a centre. Later, looked down upon from the diametrically opposite pole, only five such loops can be found (Pl. VIII, Fig. 13) because, apparently, the others are not sufficiently long to reach the hemisphere at which we are looking. The tip of a loop sometimes shows as a complete oval bend, at other times it may end in a chromatic globule, as Newton (15) found in *Tulipa*, and which he describes as a 'plasmasome', whilst other loops still may be open at their ends, a condition which Newton, too, shows in his drawings, but does not mention. When the tip is open in *Thespesia* each thread ends in a small clubbed swelling (Pl. VIII, Fig. 14). This clubbed swelling is not due to the tips having been cut by the razor; the appearance is different from an apparent blob that one does sometimes see when a thread is cut and shows its rounded sectional outline. Furthermore, Newton's drawings are apparently from uncut nuclei in which he saw the entire objects. This method of ending of the chromosome loops might be explained on the assumption that one end of a hoop at the pachytene bouquet stage broke off from the basal mass of thread whilst the other remained fixed, and the former then straightened itself out polewards away from the nucleolar pole of the nucleus, or it might be explained on the assumption that the hoop does consist of two chromosome lengths in contact at the apex as postulated by the telosynaptic theory of chromosome conjugation. The loops at this stage very much resemble those found by Gelei (11) in the developing egg of the flatworm *Dendrocoelum lacteum*, but no fusion in pairs as he describes has so far been seen. Shortly after the appearance of the lengthening loops the 'reserve' thread from which they spring becomes used up and disappears. It has already been suggested how this occurs; some seems to be drawn upon by the lengthening loops and some seems to be voided altogether from the nucleus. The two threads constituting a loop now untwist proximally and lie parallel, side by side, whilst distally they are still looped with one another. In a view of the hemisphere of the nucleus in which the nucleolus lies (Pl. VIII, Figs. 10 and 12), the loops at this stage can be counted with more certainty than at any other view or time. They appear to be eight in number. Under moderate magnification they show like the spokes of a cart-wheel radiating out from a common centre. If each ray or 'spoke' be examined under a high magnification the two parallel threads composing it can be easily made out. Furthermore, each thread itself can be seen to become irregularly vacuolated and eventually to split, at least distally, into right and left longitudinal halves (Pl. VIII, Fig. 15). Each chromosome loop is thus quadripartite.

The proximal part of a chromosome loop often remains closely twisted up as a basal chromatic projection from the central mass after the more distal parts have become divided. Indeed, the whole process in the different loops does not appear at all a synchronous one. Unsplit univalent lengths and the fine thread-like strands of split univalent lengths have characteristically earned the name 'diplonema' for this stage.

In a view of the nucleolar pole of the nucleus seen from above with the point of radiation of the threads at the centre (Pl. VIII, Figs. 16 and 17) this longitudinal splitting of the individual threads may be seen. In a view with the radiation point toward the periphery of the circular outline of the nucleus (Pl. VIII, Fig. 18) long lengths of very thin threads interspersed with thicker, as yet unsplit, ones are often apparent, traversing a diameter right across the circular outline of the nucleus. Making allowance for the difference in numbers of the spireme loops in the two organisms, this stage in *Thespesia* corresponds exactly with the process pictured by Brauer (1) of the similar stage during meiosis in the egg of *Ascaris megalocephala* var. *bivalens*.

The longitudinal splitting of the chromosome threads just described must be regarded as a precocious split indicating the line along which separation will take place at the homoeotypic division. The chromosome loops split longitudinally and the elements separate right to the extremity.

These elements show as delicate single threads with granules along their length (Pl. VIII, Fig. 19). Four such elements will show in each chromosome loop at diplonema.

Following this stage the single threads once more twist up tightly and contract. At this twisting the two elements derived by longitudinal splitting from each side of the former loop come together, and they do not again show in the prophase as separate strands. The single strands are seen in Pl. VIII, Fig. 19.

After this twisting the constrictions again loosen out, and the chain-like chromosomes (Pl. VIII, Fig. 20) are noticeably much more regular and more equal in length from node to node than they were before. Pieces now break off from the twisted chain-like strands. They are at first of a figure-of-eight (8) shape. These pieces do not seem to separate off at all synchronously. No nucleus has ever been found, in spite of much searching, in which there were a number of these pieces-of-eight, or their subsequent forms, at diakinesis. The pieces-of-eight (Pl. VIII, Fig. 21) that have been found had apparently but just separated from the chromosome chain. Each represents two loops of the chain in its final form, and in the case of the longer chains it is obvious that each is half of a chain. Eventually the pieces appear upon the equatorial plane at metaphase as thirteen globular bodies. The failure, so far, to find a diakinesis stage, and to see the loops more frequently segmenting transversely into pieces of two internodes each, is believed to be due to the interval of time occupied by these phases

being a very short one, and to the inability of fixing fluids to penetrate the hyaline membrane around the cell sufficiently quickly. Since the five longer chromosome loops give rise to two of the chromosome bodies each, it is a legitimate conclusion that the other three bodies come one from each shorter loop.

When the facts observed in this study are considered in conjunction with the observations made during the division of somatic cells, then the view that the chromosome bodies of the meiotic phase are derived from five long and three short chromosome loops becomes further substantiated. Furthermore, this derivation of the chromosome bodies explains phenomena (Youngman (20)) observed during the development of the pollen grains. A discussion can now be entered upon of the observations herein made during the prophase of the nuclear behaviour in the pollen mother-cell, and of phenomena, which now become explicable, which were seen during division in somatic cells. Some phenomena in the telophase of the nucleus during pollen grain formation in *T. populnea* now also can be explained.

DISCUSSION.

In this discussion the observations detailed in Number II of these studies will be dealt with in relation to those described in the present study.

A chromosome has been defined as 'each coherent chromatin mass, whatever be its form, mode of origin or valence, which as such enters the equatorial plate' (Wilson (18)).

When it is attempted to apply this definition in the case of the chromatin masses, as observed in *Thespesia*, it breaks down. Both thirteen and eight bodies have been found upon the equatorial plate in the same cell during pollen-grain development at different times, and the same term could therefore be applied to two sets of things.

The modern developments of genetics and cytology help us to say that a chromosome is the shortest length of chromatin-bearing spireme, never matter what its subsequent form, that behaves as an individual *both* genetically and morphologically. The term 'individual' is used in its biological sense, as defined by Huxley, for 'a single thing of a given kind'. That admits of the chromosomes consisting of many units, both genetically and morphologically, as they undoubtedly do. 'Inherent in the nature of individuality is persistent unity' (McClung (13), from whom also Huxley's definition is taken). This definition of a chromosome does not exclude fission and fusion of the chromatin masses, both of which phenomena are known to occur. Nor does it preclude lengths of chromatin-bearing spireme from differing amongst themselves in volume in the same cell, a condition that is often found.

On the basis of Wilson's definition we should be unable to say whether

the chromosomes of the microspore mother-cell in *Thespesia* were either thirteen or eight, whilst the latter definition, within the limit of our present knowledge, would confine us to eight as their number. With a knowledge of chromosome numbers based solely upon their manifestation upon the equatorial plate of somatic cells one would state that the diploid number of chromosomes in our type was twenty-six.

Further consideration then is required to enable one to decide what is the number of the chromosomes. For the sake of clearness we shall prefer, as previously, to speak of these twenty-six bodies and the thirteen haploid equivalents as the 'chromosome bodies', and not as 'chromosomes'.

The behaviour of the chromosome bodies in the pollen mother-cell prior to heterotypic anaphase may be recalled here. These bodies have been seen (Youngman (20)) to appear as thirteen globules, usually all equal in size, upon the equatorial plane of the spindle, and then to mass together at the centre of the cell, out of which mass come eight bodies to arrange themselves in a belt on the equator. On the basis of the observations of their ontogeny this reduction to eight is to be regarded as the re-association of the ten halves of the spireme-loops in five pairs, no doubt each pair belonging to the same original chromosome loop, and the three having no sister halves being left as unpartnered units. Evidence of the transverse division of chromosome loops has been seen both in somatic cells and microspore mother-cells.

The observations made during the division of the somatic cells were suggestive of a phenomenon of chromosome fission occurring. The facts that the spireme first transversely divided into a small number of pieces, which then subdivided, often upon the equatorial plate, into a larger number, and the method of refusion of these pieces into loops in the newly forming nucleus made one hesitate as to what was to be regarded here as the chromosome unit.

A study of the early prophase of the microspore mother-cell showed eight loops of a bivalent nature emerging from the spireme skein, some of which were definitely seen to give rise to pieces which by a process of transverse fission eventually formed chromatin masses. Winge (19), Punnet (16), Morgan (14), and other observers have shown in other organisms that these loops, or their subsequent forms, do act as pairs of individuals genetically, whilst many workers have shown them to be bivalent individuals morphologically. These loops then, it would seem, can be taken as the index of the number of chromosomes. Each considered from a genetic and morphological point of view consists of two balanced individuals lying longitudinally side by side. Each of these individuals is a 'chromosome'.

To emphasize a single individual, since they are so often associated in pairs, each longitudinal strand is sometimes spoken of as a 'univalent

chromosome', and the two fused strands as a 'bivalent chromosome'. Strictly speaking, it is only the univalent chromosome, and not the bivalent, that comes within our definition. It is the conception of individuality that wants to be kept in mind in thinking of a chromosome. So long as the chromatin mass acts as an individual it matters not if at times it breaks up into fragments, provided those fragments have to come together again to constitute the individual.

Transverse fission happens to the chromosome strands in *Thespesia*, but the pieces associate again at the metaphase. If the two univalent constituents of a figure-of-eight-shaped piece (that represents half of a longer strand in the microspore mother-cell) could be shown to be genetically similar to the two in the other figure-of-eight-shaped piece (i.e. the other half of the strand), that is one univalent piece in one figure-of-eight carrying the same characters as its sister half in the other figure-of-eight, then these univalent half lengths of the spireme strands would come each within our definition of a chromosome. So far, however, this has not been discussed. Until this be demonstrated then, for the reasons given, the number of chromosomes in the developing pollen mother-cell of *T. populnea* must be considered as eight, and not as thirteen.

To recapitulate somewhat and then proceed with our argument.

From the eight loops of spireme in the microspore mother-cell thirteen bodies have been seen to result. Ten of these thirteen have further been seen to be formed from five of the loops by a process of transverse division of each of these five into two. They might then be regarded as half chromosomes. The remaining three bodies when seen upon the equatorial plane at metaphase are of the same size as the other ten; indeed, they usually cannot be distinguished from amongst their fellows. They might thus be considered, from a point of view of chromatin volume, as the equivalents of half chromosomes. It will be convenient in our description to distinguish these three bodies in microspore mother-cells, or the corresponding six in somatic cells, by applying the term 'peculiar chromosome bodies' to them.

Further support to the assumption that they are half chromosomes is derived from a study of them in diploid numbers in the somatic cells. Here there was evidence that there were three pot-hook-shaped lengths, each consisting of two chromosome bodies, that, although they entered the more or less continuous spireme, did not there associate with their fellows to form mitre-shaped loops.

The other twenty bodies in the nucleus fused in pairs to produce ten pot-hook-like pieces, which pot-hooks then associated after the manner of homologous chromosomes to form mitre-shaped loops.

The appearance of six chromosome bodies at times lying apart from the rest on the equatorial plane at somatic metaphase is a further suggestion

that they were associated together in a length of the spireme by themselves.

Since the number (three) of the peculiar chromosome bodies in the functional gametes is odd, their association into three pairs (to form pot-hook-shaped pieces) in the somatic cells forces us to the conclusion that one at least, if not all of the three of these pot-hooks must consist of one peculiar chromosome body from the male gamete and one from the female gamete touching, or fused, end to end. In other words, the peculiar chromosome bodies behave as homologous chromosomes. Now each of the three peculiar chromosome bodies, on many grounds morphologically, is to be considered as equivalent to one of the other ten bodies, each of which represents half a spireme loop in the gamete, formed by transverse fission. As already stated, in volume the chromosome bodies are all the same; in appearance upon the metaphase plate they are all the same. In the somatic nucleus the peculiar bodies behave just like the other bodies when they join in pairs to form pot-hook-like lengths. Everything, in fact, suggests that they are by origin half-lengths of chromosome loops of the gamete that have lost their other half.

This very strongly supports the possibility already raised, namely, that the half lengths of each of the spireme loops, formed by transverse fission during the prophase of the nucleus of the spore mother-cell, are similar halves, not only morphologically, but genetically. In other words, it suggests that each of the five long chromosomes bears a grouping of genetic characters linearly arranged along one *transverse* half of its length, and that these same characters are again linearly arranged along the other transverse half. Such *transverse* halves thus would be similar, and in fact according to the definition given are chromosomes themselves. (The word 'transverse' is emphasized here because we must not confuse these halves with the univalent longitudinal halves of a chromosome.)

We now get a suggestion as to what phenomenon is probably underlying this fission of the chromosomes in the Malvaceae. It is a metameric repetition of parts, morphologically and genetically similar, along the spireme loops. Is it possible that the Old and New World species of *Gossypium* both contain the same number of haploid chromosome loops, but in the latter forms the metamerically similar parts are twice as numerous as in the former? Or do the New World forms contain a double set of spireme loops? As has been shown, twice the number of chromosome bodies does not necessarily imply twice the volume of chromatin when comparing different varieties. So far as can be ascertained, in the New World cottons with fifty-two diploid chromosome bodies there are no pairs of allelomorphous characters that are not also represented in the Old World forms with twenty-six. The degree or intensity of the characters of such a pair may vary, that is all.

At heterotypic division in the pollen mother-cell of *Thespesia* three chromosome bodies have been seen not to divide but pass to one pole of the spindle in an undivided state (Youngman, 12). Why they should not divide like the other ten is not clear. It could be that the first reduction division be equational and that the reassociated halves of the five chromosomes simply parted company, which could not occur in the case of the peculiar three. It has, however, not been possible so far to confirm this point, and anyhow it would not seem to be the whole explanation. At the completion of the telophase of the heterotypic division, the chromosome bodies have been seen to appear as five cross-shaped and three hatchet-shaped forms in one sister nucleus, and as five cross-shaped forms only in the other. At the homoeotypic division the three peculiar chromosomes again fail to divide, the result being one nucleus with thirteen chromosome bodies and three with ten each, in the pollen tetrad (Youngman, 20). If the suggestion that the first division were equational were solely the reason for the non-division of the three peculiar bodies it might be expected that they would show at telophase as cross-shaped bodies like the other five, which they do not. It is possible that there is a traumatic factor influencing the behaviour of the three bodies at this stage. This is perhaps why they do not open out into a cross at the telophase but show the hatchet-shape. It may be that the chromosomes have not recovered, as it were, from the effect of loss of half their chromatin. The reasons for these suggestions may be considered more fully. It was thought that the condition of the only one pollen grain with thirteen chromosome bodies in each tetrad was perhaps exceptional in *T. populnea*, and considerable search was made to try and discover thirteen bodies at each pole of the heterotypic spindle, but so far without success in the material available.

The material investigated may show pathological conditions during microspore formation and these conditions may be the result of past evolutionary changes in the nucleus.

In view of the fact that twenty-six chromosome bodies are general in the somatic cells of *T. populnea* one of two things seems certain, either pollen mother-cells do exist with thirteen chromosome bodies all dividing at heterotypic division and the phenomena herein described do not give rise to functional pollen grains, or else the single cell of a tetrad with a nucleus containing thirteen chromosome bodies has in some way undergone the equivalent of the reduction division. The first suggestion seems the more likely. The events observed during pollen-grain formation then would be pathological, but not without interest in suggesting a way in which a change has taken place in nuclear structure.

In the Old World forms of *Gossypium* thirteen bodies come upon the equatorial plane at heterotypic metaphase and at the following telophase (Pl. VIII, Fig. 22) they have been found to give thirteen bodies at each

pole, which again come on to each of the homoeotypic spindle planes as thirteen bodies (Pl. VIII, Fig. 23). This has been found in more than one species of *Gossypium*.

Thespesia and *Gossypium* have without doubt originated close together from some ancestral stock. They may, or may not, be in a common line of descent, one having descended from the other through intermediate, perhaps now lost, forms. Is it not possible that both have come from an ancestral line in which there were eight equal chromosome loops at prophase in the nucleus of the spore mother-cell, and that these loops having the property of segmenting transversely into two, lost half their chromatin in the case of three of them? Is it possible that the phenomenon of 'cyto-mixis', seen to be a general one amongst these plants, is the stage at which this chromatin is expelled from the nucleus? That 'cytomixis' occurs in other plants does not invalidate this suggestion.

As has already been mentioned, forms with eight haploid chromosomes occur amongst the Sterculiaceae, an order of plants nearly related to the Malvaceae.

It is quite conceivable that *Thespesia* is the stock, or nearer the stock, in which three chromosome loops in the nucleus first suffered, in some way, and for some reason that we do not at present understand, the loss of half their chromatin substance.

The failure, in the material investigated, of three chromosome bodies to divide in the developing gametophyte may be a trait due to this past 'wounding' as it were. In both *Thespesia* and *Gossypium* experiment shows that a large number of the pollen grains that find their way to the stigmas do not function in fertilization.

SUMMARY.

1. At early prophase the nucleus of the pollen mother-cell has its periphery traversed by thin chromatic strands. Soon a distinct longitudinal doubleness in some of the threads on the nuclear surface is seen. Amongst the threads that do not show doubleness some are thinner than others. It is concluded that the thicker threads consist of two thinner threads longitudinally fused.

2. When the threads have paired generally the spireme mass leaves the periphery for the inside of the nucleus. The longitudinally double thread there balls up at one side of the nucleus.

3. The spireme next opens somewhat from a compact mass. From out of this mass horse-shoe-shape hoops of thick, darkly chromatic threads emerge. The hoops become less regular in their curvings and the spireme is thick, showing signs of being longitudinally double.

4. In a later stage eight looped projections emerging from a basal mass are seen in side view. Two of these loops are of a characteristic

form, and shorter than the rest, and there is reason to believe that there is a third loop which remains short. Next each loop appears to twist up proximally into a cord-like strand. In a surface view of the hemisphere of the nucleus in which they originate the eight loops can be seen radiating from a centre.

5. From the synzesis stage onward the phenomenon of 'cytomixis' is of constant occurrence. In this phenomenon some of the chromatic material is voided from the nucleus.

6. The eight loops next split along their length, the length thus being double, so that each loop now consists of four strands in a group.

7. The loops twist up again into a tight strand. At this stage it appears that only five of the strands reach the pole of the nucleus opposite to that of their origin. Later they open somewhat from their twistings into loops with a more equally internoded condition.

8. Each of the five long loops divide transversely into two lengths of a figure-of-eight shape.

9. No diakinesis stage has been seen. This is apparently due to the fact that the cell is now surrounded by a thick hyaline membrane which does not allow entry of the fixing solution sufficiently quickly.

10. The products of the eight loops appear upon the equatorial plane at metaphase as thirteen chromosome bodies, ten of which have been derived from the five longer loops by transverse fission of each into two, and it is legitimate to assume that the other three have come one from each of the other three loops.

11. During microspore formation in *T. populnea* both thirteen and eight bodies have been seen upon the equatorial plate at different times in the same cell.

12. If a chromosome be considered as the shortest length of chromatin-bearing spireme that behaves as an individual, both genetically and morphologically, then the possibility of a fission or a fusion of chromatin masses upon the equatorial plate is not excluded from chromosome behaviour. This definition determines under what condition a chromatin mass upon the equatorial plate may be regarded as a chromosome.

13. On the basis of observations upon the ontogeny of the thirteen bodies seen upon the equatorial plate during microspore formation in *T. populnea* their reduction to eight in number is to be regarded as the re-association in pairs of the ten halves of five of the spireme loops, plus three unpartnered pieces (to be considered as the equivalents of halves) of three other loops.

14. From the behaviour of the chromosome bodies during mitosis in the nucleus of somatic cells it is suggested that the two half lengths of each of five of the spireme loops in the microspore mother-cell are similar morphologically and genetically.

15. The material investigated may show pathological conditions during microspore formation, and other conditions with thirteen chromosome bodies all dividing at heterotypic division, although not found, probably do occur in *Thespesia*, since twenty-six chromosome bodies are general in the nucleus of somatic cells. The pathological condition, however, possibly indicates the line along which an evolutionary change has occurred in the nucleus.

16. In microspore formation in *Gossypium* thirteen bodies come upon the equatorial plate at heterotypic metaphase, and in the two following divisions regularly give thirteen chromosome bodies at each pole of the spindle.

17. It is suggested that both *Thespesia* and *Gossypium* may have descended from an ancestral line of plants in which there were eight equal chromosome loops in the nucleus of the spore mother-cell.

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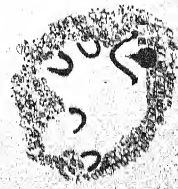
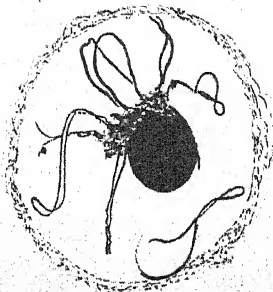
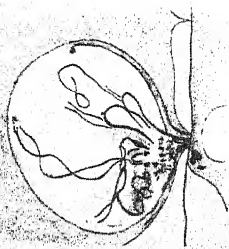
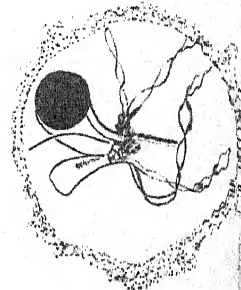
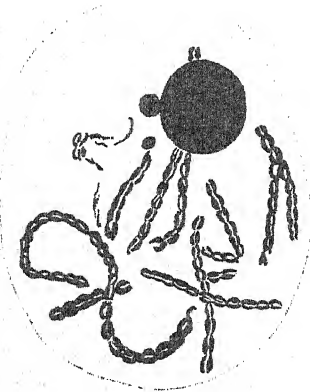
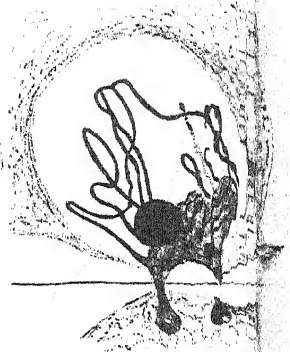
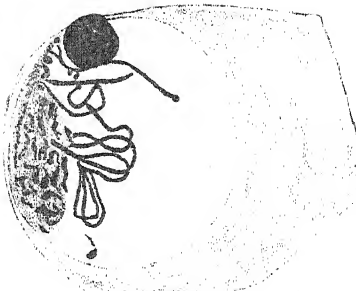
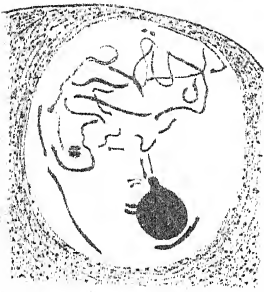
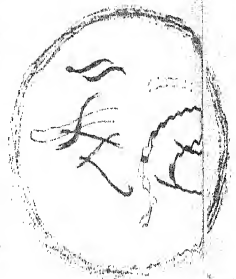
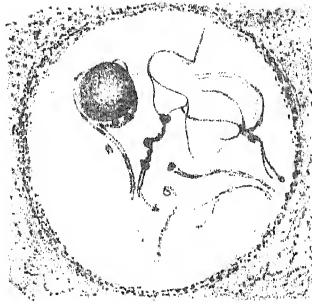
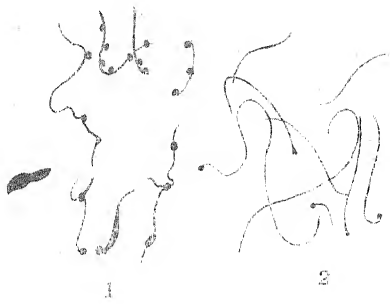
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EXPLANATION OF PLATE VIII.

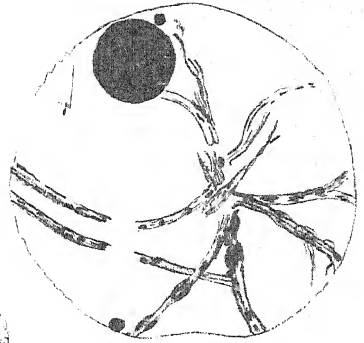
Illustrating Dr. W. Youngman's paper on Studies in the Cytology of the Hibisceae. III.

- Fig. 1. *Thespesia populnea*. Spireme as seen on the surface of a nucleus at early prophase. $\times 3,040$.
- Fig. 2. *Thespesia populnea*. As Fig. 1 but slightly later. $\times 3,040$.
- Fig. 3. *Thespesia populnea*. Section of a nucleus showing its spireme at a later stage than Fig. 2. $\times 1,520$.
- Fig. 4. *Thespesia populnea*. As Fig. 3. $\times 1,350$.
- Fig. 5. *Thespesia populnea*. Section through a nucleus at synizesis. $\times 1,520$.
- Fig. 6. *Thespesia populnea*. Nucleus at synizesis. $\times 1,520$.
- Fig. 7. *Thespesia populnea*. Nucleus of one cell showing chromatin material passing through the cell-wall into the next cell. $\times 1,520$.
- Fig. 8. *Thespesia populnea*. Section through nucleus showing the nucleolus and some of the spireme. At about the stage of Fig. 7. $\times 2,280$.
- Fig. 9. *Thespesia populnea*. Section of nucleus at same stage as Fig. 7, but viewed in a direction at right angles to that shown in that figure. $\times 1,520$.
- Fig. 10. *Thespesia populnea*. Nucleus showing eight spireme loops and a nucleolus. $\times 1,520$.
- Fig. 11. *Thespesia populnea*. Portions of two cells with some eight twisted spireme loops in the nucleus of one and cytomixis taking place. The nucleus is seen in a direction at right angles to that of Figs. 10 and 12. $\times 1,520$.
- Fig. 12. *Thespesia populnea*. As Fig. 10. $\times 1,520$.
- Fig. 13. *Thespesia populnea*. The pole of a nucleus showing the tips of five loops. $\times 1,520$.
- Fig. 14. *Thespesia populnea*. Section of a nucleus apparently showing the ends of some of the loops at a later stage than Fig. 13. $\times 2,280$.
- Fig. 15. *Thespesia populnea*. Section of a nucleus showing some of the spireme loops radiating from a central point. $\times 2,800$.
- Fig. 16. *Thespesia populnea*. Section through a nucleus at diplonema. $\times 2,280$.
- Fig. 17. *Thespesia populnea*. Nucleus at diplonema. Possibly later than Fig. 16. Eight groups of strands are seen. $\times 2,280$.
- Fig. 18. *Thespesia populnea*. Section through nucleus at diplonema. Seen at right angles to the direction of Fig. 16. $\times 2,280$.
- Fig. 19. *Thespesia populnea*. Portions of apparently single strands of spireme at diplonema. $\times 4,560$.
- Fig. 20. *Thespesia populnea*. Portion of a spireme loop at a later stage than diplonema. $\times 2,280$.
- Fig. 21. *Thespesia populnea*. A portion transversely segmented from off a spireme loop. $\times 3,400$.
- Fig. 22. *Gossypium Stocksii*. The chromosome bodies at opposite poles of the spindle at telophase of the first meiotic division in the development of the pollen grains. The drawings are from consecutive sections which are transversely oblique to the long axis of the spindle. $\times 2,280$.
- Fig. 23. *Gossypium neglectum* var. *roseum*. Microspore development. Section of the two-celled stage within its gelatinous envelope. The cells show their nuclei with the chromosome bodies on the equatorial plane preparatory to the second meiotic division. The spindles in this case lie parallel to one another in the same plane instead of, as more often, at right angles to one another. $\times 2,280$.

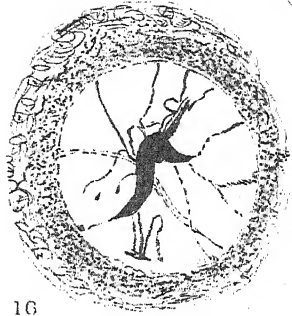




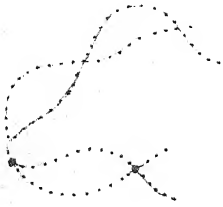
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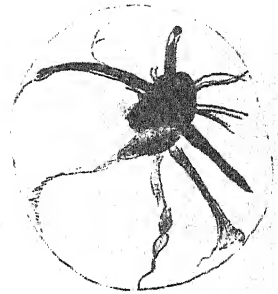
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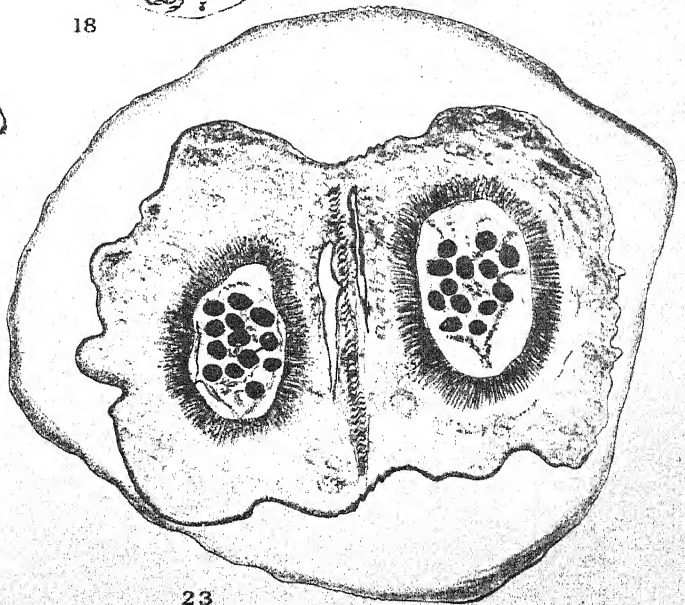
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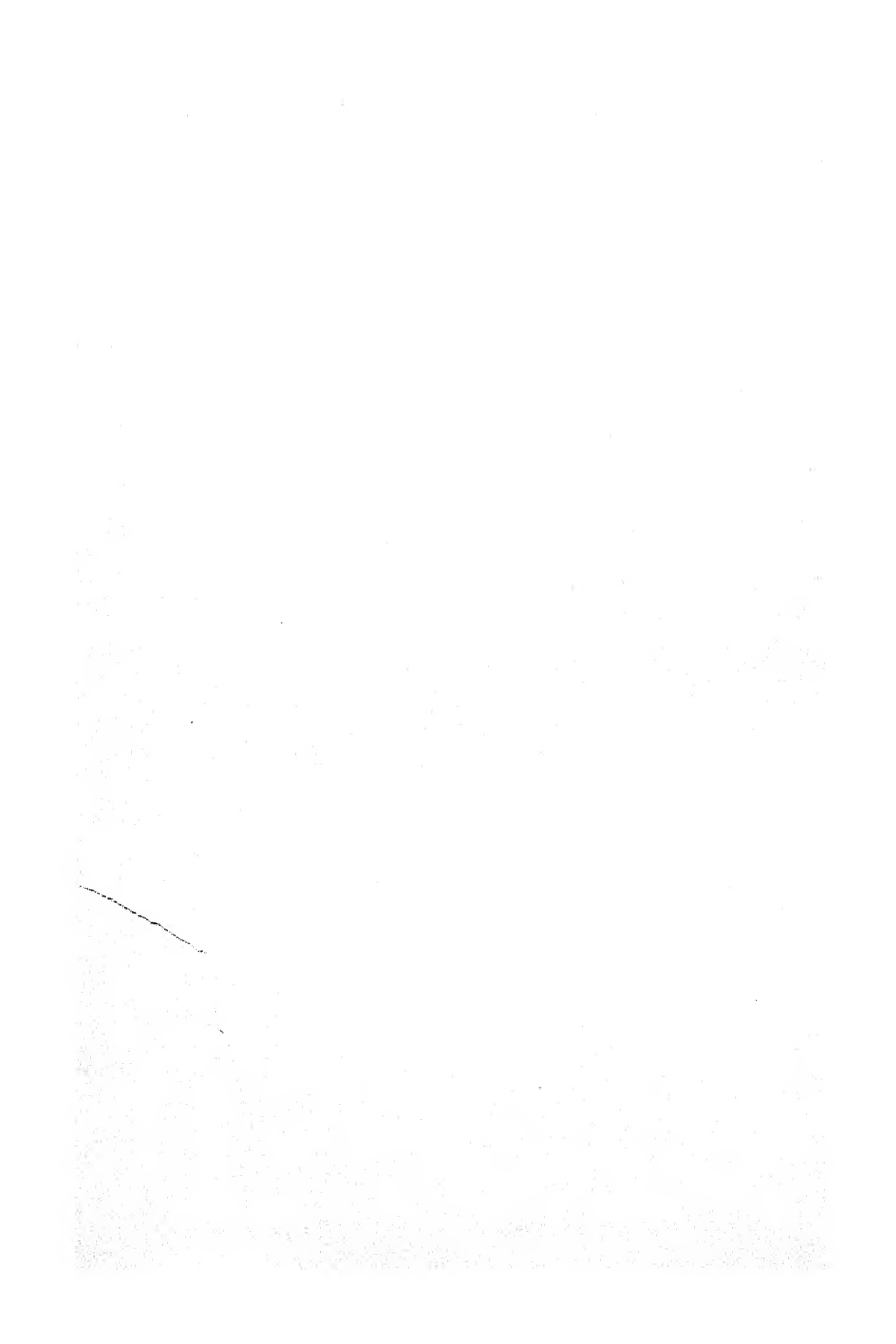
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23



A Hybrid *Daphne* (*D. petraea*, Leybold \times *D. Cneorum*, L.).

BY

ARTHUR W. HILL.

With three Figures in the Text.

A HYBRID *Daphne* (*D. petraea* \times *D. striata*) was described and figured under the name *Daphne* \times *Thauma* by the late Mr. Reginald Farrer in the *Gardeners' Chronicle*, ser. 3, III, of July 13, 1912, pp. 22, 23, Fig. 9.

This hybrid was found by Mr. Farrer on the Cima Tombéa in August 1911, and the photograph shows the plant he had collected and established in his garden at Ingleborough. Unfortunately no herbarium specimens were made of this interesting hybrid nor can the plant now be found at Ingleborough.

Farrer suggests that this hybrid is the only case recorded of a hybrid *Daphne*, but Dr. Karl v. Keissler in his monograph on the genus *Daphne* (1) refers to several 'bridging forms' between *D. oleoides* Schreb. var. *glandulosa* Bertoc. and *D. alpina* L., which may well be hybrids, and under *D. striata* and *D. Cneorum* varieties are recorded which experiment might prove to have arisen as the result of hybridization. Under *D. petraea* (*D. rupestris* Facch.), no variants are mentioned, so it is of interest to record the finding of what appears to be an undoubted hybrid between *D. petraea* and *D. Cneorum* in a valley on the W. side of the Lake of Garda in May of this year by Mr. W. Scott Henderson, when we were wandering together in search of plants.

The valley was almost carpeted with *D. Cneorum* in full flower and on the steep limestone cliffs beautiful cushions of *D. petraea*, thickly covered with soft pink flowers, were in considerable numbers. In several places *D. Cneorum* occurred both at the foot and at the top of the limestone bluffs on which *D. petraea* was growing, and sometimes the looser-growing plants of *D. Cneorum* overhung the cliffs, and were almost in contact with the rosettes of *D. petraea* growing in clefts on the rock faces. It was in such a situation, at the upper edge of a vertical limestone bluff, that the hybrid plant was found.

The principal differences, besides the habit, between *D. Cneorum* and *D. petraea* are in the leaves and bracts, and in the number of flowers in the inflorescence. *Daphne Cneorum* and *D. petraea* have been so well described by Keissler (2) and Leybold (3) that it is unnecessary to give full descriptions again. *D. Cneorum*, however, differs from *D. petraea* in its lax, corymbose, more or less erect, branches, flat usually emarginate leaves with a prominent midrib and often slightly revolute margins, and

with green leaf-like bracts, slightly hairy on the margins, equal or almost equal in length to the perianth tubes of the flowers. There are usually 8–10 flowers in the capitula, and the perianth tube is densely covered with closely adpressed hairs. In *D. petraea* the stems are much branched, and the short tortuous branches are only very sparsely puberulous. The spatulate leaves are thick and fleshy, apiculate, sulcate, and more or less V-shaped, the midrib not being noticeable in the fresh specimens. The bracts are scarious and ciliate, and only about quarter the length of, or less than, the perianth tube. The capitula consist of only 3–5 flowers, and the perianth tube is covered with soft, somewhat woolly, spreading hairs, while the segments are much broader than in *D. Cneorum*, have incurved margins and are pale pink in colour.

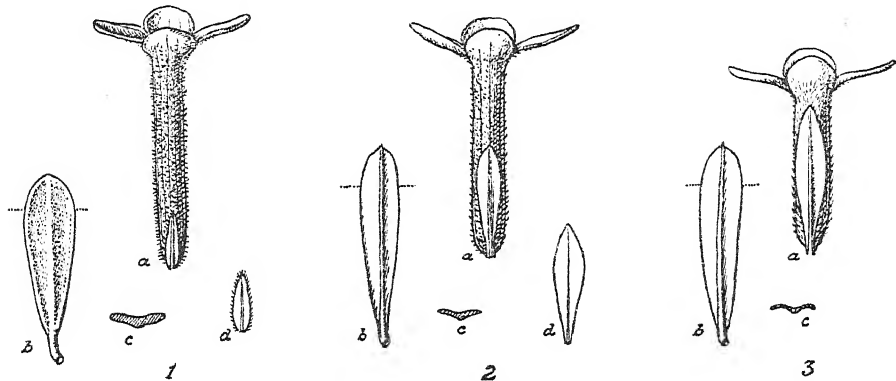
The distribution of the stomata on the underside of the leaf differs in the two species, for in *D. Cneorum* they cover the whole of the under surface with the exception of the narrow prominent midrib, while in *D. petraea* they are confined to two bands on either side of the indistinct midrib, leaving a clear somewhat hyaline margin and a clear median area free from stomata. The hybrid was easily recognizable by being remarkably intermediate in character between the two parents. The stems are elongate and finely puberulous, ascending as in *D. Cneorum*, which species it approaches more nearly in its general appearance. The leaves are spatulate and apiculate, more fleshy than in *D. Cneorum*, sulcate, with the midrib fairly prominent on the under surface. As in *D. petraea*, however, the margins of the leaves are free from stomata. The bracts are green, as in *D. Cneorum*, but are glabrous, and are not more than half as long as the perianth tube. The hairs of the perianth tube are more or less adpressed and are less spreading than in *D. petraea*. The flowers are of a soft pink colour, as in that species, and there are 6–7 flowers in the capitula; the segments are usually flat as in *D. Cneorum*, and the anthers, which are small, are also situated in the throat of the perianth tube.

As the leaves of the two species differ considerably in external appearance and in the arrangement of the stomata, it seemed desirable to examine their structure and compare them with those of the hybrid by means of transverse sections, and I am greatly indebted to Mr. F. N. Howes and to Dr. Metcalfe for having cut and examined the sections and supplied the following notes:

A comparison of the anatomy of the leaves of *Daphne petraea* Leyb. and *D. Cneorum* L., and the hybrid between them, failed to discover any definitely contrasted characters in the two parents which were represented in an intermediate form in the hybrid. There were, however, certain characters which tended to differ in the two parents and to be intermediate in the hybrid although no hard and fast line could be drawn between them.

The cells of the upper epidermis varied in size in all cases, and large

cells containing mucilage were frequently present between the epidermis and the mesophyll. However, in surface view whereas the lateral walls of the upper epidermis of *D. Cneorum* were markedly wavy in outline, those of *D. petraea* were less wavy and those of the hybrid tended to be intermediate in character. The actual cells of the upper epidermis of *D. petraea*



FIGS. 1-3. 1. *D. petraea*. 2. Hybrid. 3. *D. Cneorum*. a. Flower with bract. b. Leaf. c. Leaf (in cross section). d. Floral bract. All $\times 3$. (G. Atkinson Del.)

and the hybrid were larger and more similar to one another than those of *D. Cneorum*. A further point of similarity between *D. petraea* and the hybrid, was the presence of groups of one to several large cells with thick pitted walls scattered in the mesophyll. These cells, which in structure resembled stone cells of a rather unusual type, were absent in *D. Cneorum*.

Another interesting feature was found in the arrangement of the stomata. In *D. Cneorum* the cuticle is greatly thickened around the stomata so that the guard cells are situated at the base of a deep cavity. In *D. petraea*, on the other hand, this peristomatal thickening is very little developed, whilst in the hybrid it tends to be intermediate in character between that shown by the parents. However, measurements of this thickening in large numbers of stomata from all the plants show that this character varies considerably within both the parent species themselves, as well as in the hybrid.

The anatomical characters, therefore, support the view that the plant is a hybrid between *D. petraea* and *D. Cneorum*.

LITERATURE CITED.

1. KEISSLER, K. U.: Die Arten der Gattung *Daphne* aus der Section *Daphnantes*. Engl. Bot. Jahrb., xxv, pp. 29-125, with Plates I-IV, 1898.
2. ———: Ibid., pp. 77 and 78.
3. LEYBOLD: Flora, p. 81, 1853; p. 346 with Fig. xiii, 1855.

Conjugation in *Spirogyra*.

BY

HAZEL SAUNDERS.

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With eight Figures in the Text, and four Diagrams.

I. INTRODUCTION.

THE lacing together of the filaments of *Spirogyra* during conjugation is usually described in the following way: 'two threads become ranged parallel to one another and their opposing cells develop finger-like protrusions which grow towards each other till they meet and fuse; after this the separating wall breaks down so that an open tube (the conjugation canal) is established (4, p. 226). In the second edition (1927) of the book just quoted, this statement is modified to read 'two threads become ranged parallel to one another and their opposing cells develop finger-like protrusions, the tips of which are in contact with one another; after this the separating wall breaks down so that an open tube (the conjugation canal) is established'. This more guarded statement was conditioned by the work of Czurda (2) published in the interval between the two editions, and it was at the suggestion of Professor Fritsch that this corroborative investigation was undertaken.

Descriptions of the conjugation process such as that quoted at the outset, are ultimately derived from the records of De Bary (3), Haberlandt (5), Klebs (7), and other early workers. Their accounts of the early stages of conjugation are, however, very scanty. Although it is quite definitely stated that papillae from opposite cells grow towards one another and meet, no details as to the time taken for this process or of the distance apart of the filaments at the beginning of conjugation are usually given. Overton (11, p. 69), Haberlandt (5, p. 2), and Klebs (7, p. 230) all remark on the certainty with which the papillae meet and their restriction to the sides of the filaments facing each other. That these facts puzzled them shows that they had no conception of the filaments at first lying in direct contact, when mutual stimulation at corresponding points on the filaments (which they assume) could so easily be explained.

Czurda (2, p. 443), in amplifying and corroborating certain observations of Hemleben (6), describes an essentially different method of lacing together of the filaments. In the earliest stages of conjugation he finds the filaments united in pairs or bundles; opposing protuberances are *then* put out from the adjacent sides of the two filaments. These protuberances are thus in contact from the first moment of their formation and, as a result of their elongation the filaments are gradually pushed apart (cf. West and

Fritsch, 13, p. 233). These observations were made on living filaments in their natural habitat, for Czurda found that in the species he examined conjugation set in simultaneously throughout the wads, so that the stage reached by any one filament pair represented the stage reached by all the filament pairs in the wad (2, p. 441).

The following investigation of conjugation in *S. weberi*, Kütz., *S. varians* (Hass.), Kütz., var. *scrobiculata*, Stockm., and *S. cataeniformis* (Hass.), Kütz., carried out at the Royal Holloway College, affords further evidence that the sequence of events just described is probably the usual one in all species of *Spirogyra*.

II. METHOD OF MAKING AND RECORDING OBSERVATIONS.

In the three species examined, the early stages of conjugation were destroyed by fixation in all the usual fixatives (medium chrom-acetic acid, Bouin's fluid, alcohol, and formalin), although material fixed and stained in picronigrosin and stored in dilute glycerine was more satisfactory. Observations on such fixed material were, however, not nearly so conclusive as those made on the living alga. Frequent collections followed by immediate examination were necessary, for conjugation was not found to occur in filaments removed from their natural habitat, although it tended to continue in filament pairs in which it had already started. Czurda's method (see above) could not be applied to any of the three species, since conjugation was not simultaneous in the wads, so that any one filament pair could not be taken as typical of the stages reached by the whole wad.

During 1928-9 a number of pools and ditches harbouring *Spirogyra* were kept under frequent observation for the first signs of conjugation. In 1929 three species conjugated, the periods following conveniently one after the other, *S. weberi* was found in conjugation from April 19th to May 8th and *S. varians* from May 16th to June 4th, both occurring in ditches in the neighbourhood of the college. *S. cataeniformis* conjugated during June in a pond in the grounds of the college. In each case material was collected at least once and sometimes twice a day and examined immediately after collection.

Fortunately the material of *S. weberi* and *S. varians* grew in water free from detritus which, when present, tends to coat the conjugating filaments, so that their limits are often difficult to see. The pond with *S. cataeniformis* contained a particularly large amount of silt and both the individual cells and the conjugation tubes were often quite obscured by a rich coating of detritus. Some filament pairs, however, showing early stages of conjugation, were found among a deep green wad of almost entirely vegetative material and they were quite clean, presumably because the wad was still intact and its threads not yet in intimate contact with the silted water.

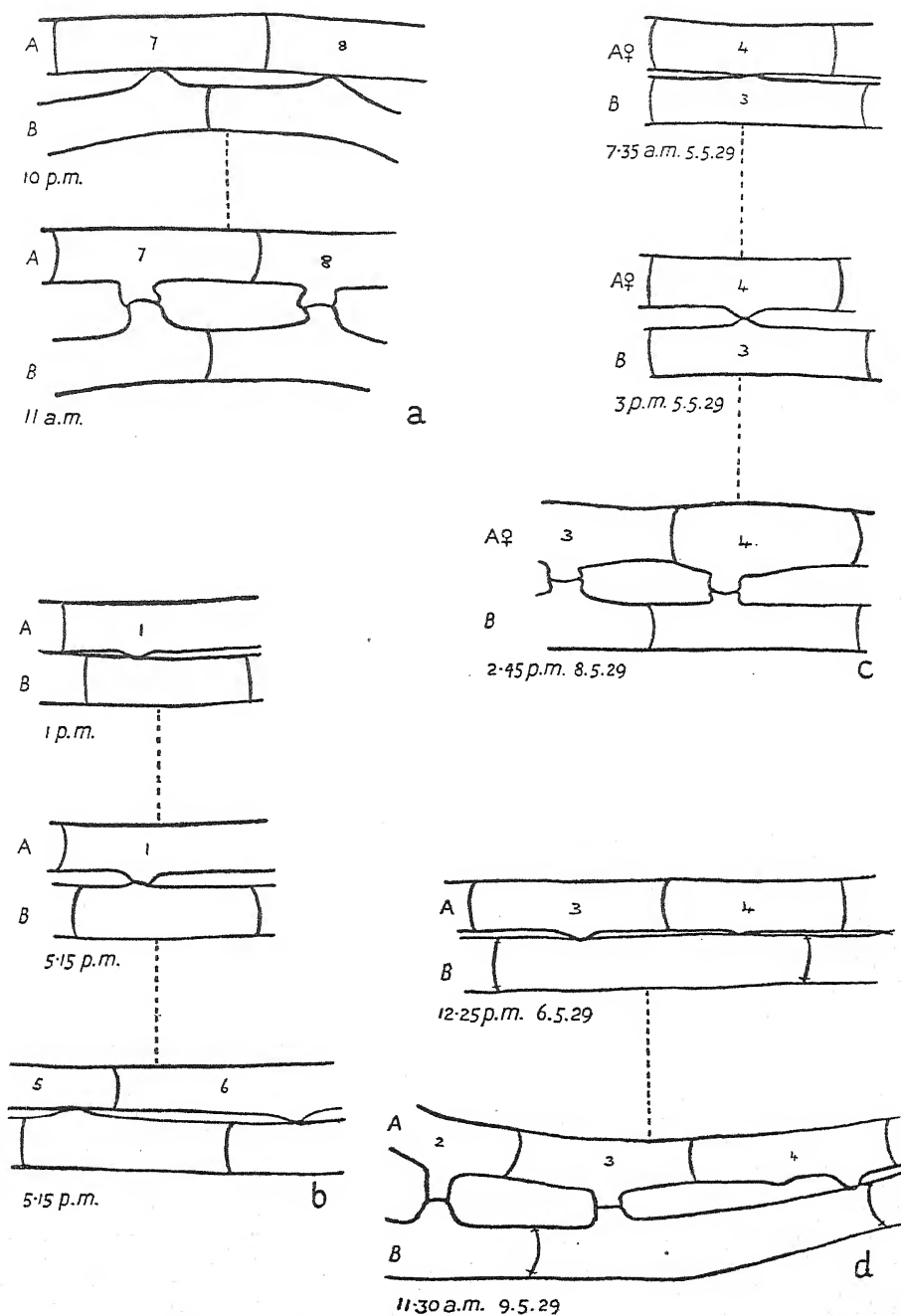


FIG. 1. Conjugation stages in *S. weberi*. a, b, c, d, successive stages of conjugation in different pairs of filaments (all \times about 315). See footnote on p. 236.

Fresh material of *S. weberi*, the first species to be found in conjugation, was critically examined for the rare early stages, i. e. those in which the filaments of a pair were not separated by a wide space as they were in later stages. Such early stages, several of which were drawn (Fig. 2 *a, d, e*), conclusively showed that the papillae were always in contact, and that when they were short, the filaments were only slightly separated from each other, whereas, when they were longer, the filaments were further apart. This implies that, if the filaments are not in actual contact when the papillae are first put out, they must be very close together.

But convincing evidence of the mode of conjugation is only to be obtained by following the process through all its stages. In *S. weberi* the early stages showed no further development when continuously irrigated. It was found, however, that constant illumination with artificial light of 30 C.P. acted as a stimulus to conjugation (cf. Klebs, 7, p. 242) and by employing special methods of culture (see below) the process was successfully followed in all its stages in a considerable number of filament pairs. Similar methods were used for the other two species, but in the case of *S. varians* artificial light seemed to hinder rather than to further conjugation.

The cultures were set up in the following way: immediately after collection, a small portion of the conjugating material was snipped out of the wad with a pair of scissors and gently teased out on a slide with glass needles in a drop of the natural water in which it was living. No cover-slip was placed over the material during its observation under the microscope. Any two filaments in contact but showing no indication of papilla formation may have come together either by chance, or because of being glued together as a preparation for conjugation. By agitating the water or by moving the projecting end of one of the filaments, those merely in chance contact immediately separated or glided along one another, whereas filaments glued together could not be separated by this means. This method of identifying conjugating filaments proved to be infallible.

An easily recognizable portion of a filament pair, A and B, in an early stage of conjugation was selected; the cells of A were numbered, and the changes in them with regard to those of the corresponding filament B were noted down at each period of observation. The data thus obtained were diagrammatically represented (cf. Diagrams I, II, and III; for explanation, see p. 250) and, where time allowed, outline camera lucida drawings of portions of the filament pairs were made (Figs. 1–8).¹

¹ In these figures A may be either the male or the female filament; the sex is indicated, if known, by later differentiation. The observations follow on through the day, and from day to day, and all the times given refer to Summer Time (an hour later than Greenwich time). The date is given where there may be doubt as to which day the time refers to. All drawings have been made with camera lucida; slight discrepancies in the drawings are due to the depth of the water that had to be focussed through.

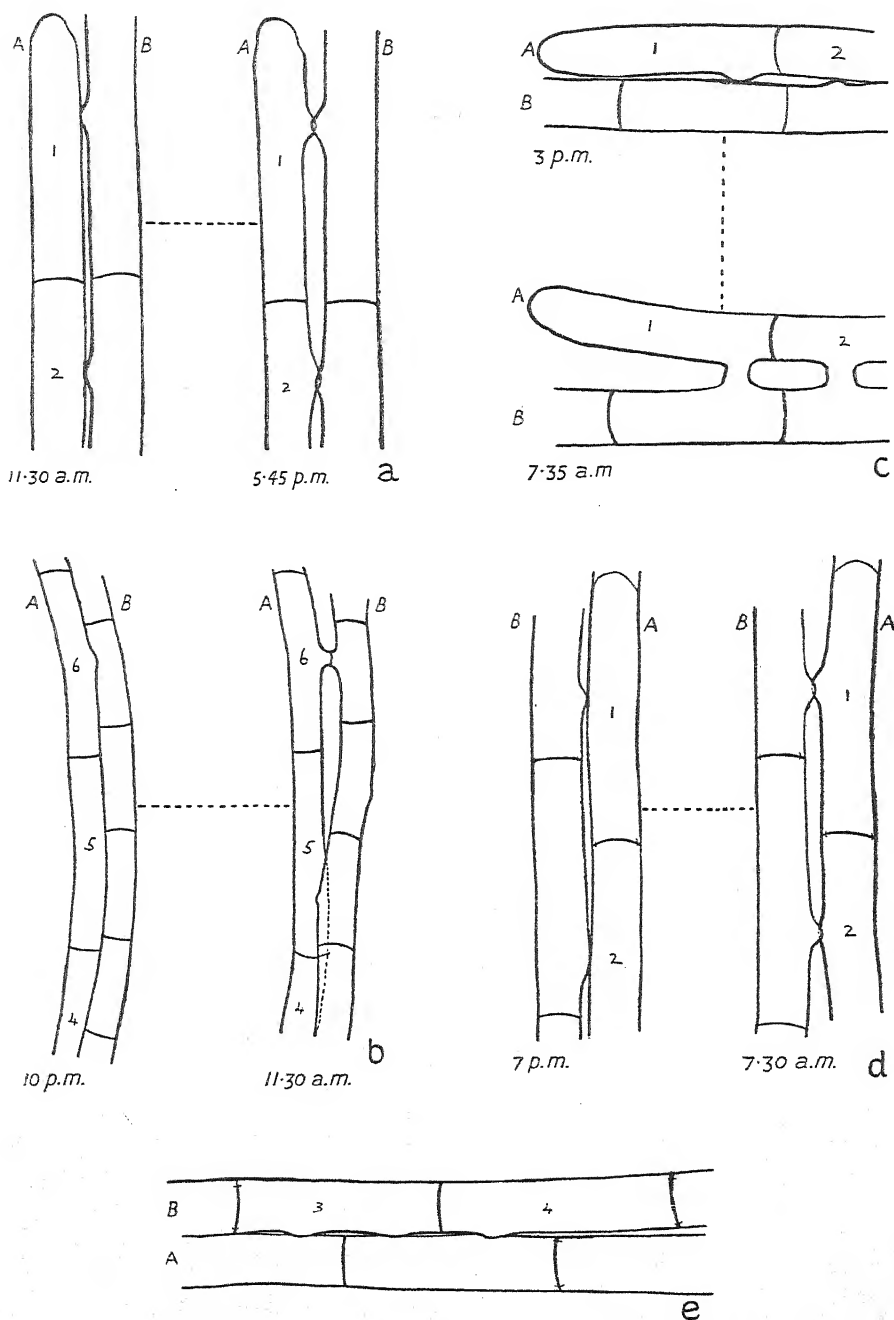


FIG. 2 *a-e*. Conjugation stages in *S. weberi*. *a, b, c, d*, successive stages of conjugation in different pairs of filaments (*a, b, d, e* \times about 315; *c* \times about 200). See footnote on p. 236.

After each observation the slide was placed in a covered Petri dish lined with wet filter paper and, thus treated, the material remained healthy and development proceeded until the characteristic swollen female cells were formed. This happened about sixty hours after contact in *S. weberi* and about thirty-six hours after contact in *S. varians*. In *S. cataeniformis* conjugation tubes were rarely formed in cultures and the process was extremely slow. In this species the conjugating filaments were relatively short and tended to break up during conjugation, so that the one under observation cannot be recognized with certainty unless all other filament pairs were removed from the culture. This was accomplished with the aid of a dissecting microscope and in a few of the cultures thus obtained further development took place. In *S. varians*, where it was found that the growth of the papillae was comparatively rapid, cultures were kept permanently on the stage of the microscope, a watch-glass being placed over the slide between the observations and the drop of liquid being frequently replenished with distilled water.

Photographs of the early stages of conjugation were also attempted, but successive photographs of the same pair of filaments were only obtained by good luck, for they had to be taken through a drop of water of varying depth, since no cover-slip could be placed over the material. The exposure time was reduced as much as possible by using a Point-o'-lite lamp, but the filament pairs were frequently pushed out of place during the exposure by motile organisms present in the drop. In many cases the filaments died or were lost or had altered their position so as to overlap, so that a second photograph could not be taken. A few were nevertheless successful and provided additional evidence for the early stages of the conjugating process.

III. THE WALL OF *SPIROGYRA*.

The wall of *Spirogyra* is usually described as consisting of an inner cellulose layer surrounding the entire cell cavity, and an outer layer continuous over the exposed surface of the filament. This outer layer has been referred to under various names, viz. Hüllhaut (de Bary, 3, p. 1), Cuticula or Cuticularschicht (Strassburger, 12, p. 68, Oltmanns, 10, p. 90), Gallertscheide (Klebs, 8, p. 334). When immersed in indian ink, methyl violet, or methylene blue this outer layer, in *S. varians*, is seen to be composed of two distinct regions, viz. (*a*) a firm layer directly in contact with the cellulose layer and marking the visible outer limit of the wall in unstained filaments and, beyond this, (*b*) a mucilage sheath of varying width which is invisible until stained (Fig. 3, *c*). These two outer layers correspond respectively to the cuticle (Cuticula) and mucilage sheath (Schleimschicht) described by Benecke (1, p. 457). In the narrower

species *S. weberi* and *S. cataeniformis* a very thin mucilage sheath, although not directly demonstrable, is probably likewise present since these filaments show a clear outline when mounted in indian ink.

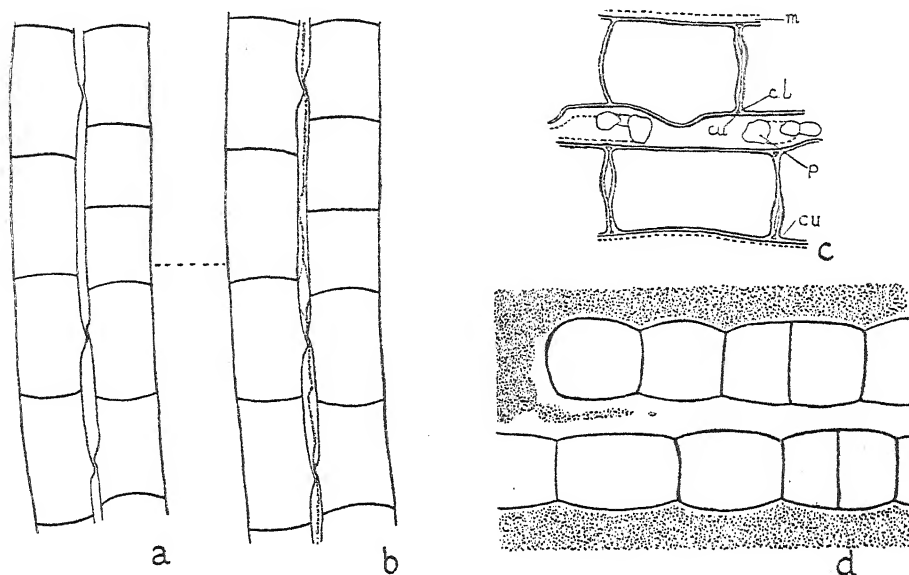


FIG. 3. Conjugating filaments of *S. varians*. *a*, filament pair in 0.1 per cent. ruthenium red. *b*, the same after addition of picronigrisin; the mucilage between the filaments has become apparent. *c*, filament pair after immersion in methylene blue. *d*, filament pair after immersion in indian ink; the mucilage has become apparent. *cl.*, cellulose layer; *cu.*, cuticle; *m.*, mucilage; *p.*, particles embedded in mucilage (*a*, *b*, *d* \times about 200; *c* \times about 315). See footnote on p. 236.

IV. METHOD OF PAIRING FILAMENTS.

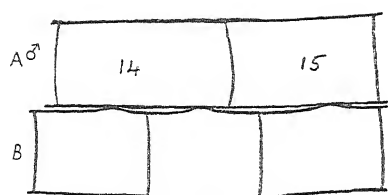
In agreement with Czurda's observations (2, p. 443) the filaments have been found to come together in firmly adhering parallel pairs or bundles at the beginning of conjugation (Figs. 5*d*, 6*a*, 8*a*; Diagrams I, II iii, III iii, v). In *S. cataeniformis* bundles are more frequently met with than pairs of filaments. The firm connexion must be due to some alteration in the mucilage sheath. Czurda (2, p. 443), in describing the early stages of conjugation in *S. setiformis* (Roth.), Kütz., speaks of 'eine ausgesprochene Verkittung der Fäden mittels der verquollenen, undeutlich geschichteten primären Wandschicht (Hüllhaut, de Bary; Cuticula oder Cuticularschicht, Strassburger, Oltmanns; Gallertscheide Klebs.)'. In the three species here investigated, however, the filaments lie in very close contact and are cemented together by the invisible mucilage sheath only, and no change can be observed in the cuticle, which forms the visible limit of the wall during conjugation. No swelling of the wall as reported by Czurda has been noted and the mucilage sheath between the

filaments cannot usually be seen (Fig. 3 *a*). In *S. varians*, however, where the conjugating filaments sometimes appear slightly separated, one has proof of the presence of the connecting mucilage, which is presumably formed from the mucilage sheath (Figs. 3 *d*, 4 *b*; Diagram II, iv).

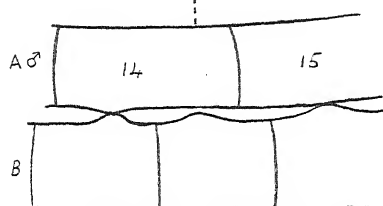
Immersion in indian ink or treatment with picronigrosin shows that, when the filaments are very slightly pushed apart by recently formed papillae, the intervening space is occupied by mucilage (Fig. 3 *b*), but this is not the case in later stages when the filaments have become more widely separated. Filaments firmly glued together by mucilage, separate when treated with chlorzinc-iodine, presumably owing to alterations in the adhesive nature of the mucilage. Addition of dilute glycerine to such filaments shows the cuticle still intact, and this is especially clear over the now separated transverse septa.

The clinging together of the filaments of *Spirogyra* is possibly an analogous phenomenon to the adhesions which, together with geniculations, were interpreted by de Bary (3, p. 14) as early stages of conjugation in *Sirogonium* and in species of *Mougeotia* belonging to the section *Craterospermum*. He noted (3, p. 23) these phenomena in other species of *Mougeotia*, where they were found to have no connexion with conjugation. De Bary regarded the cementing substance as being formed by a strongly developed portion of the 'hüllhaut'. Lloyd (9, p. 105), dealing with the adhesions and geniculations present in *S. longata* (Vauch.) Kütz., at the time of conjugation, found that they had no direct relation to this process. According to him a definite cementing material is usually only apparent after staining, when it appears 'quite distinct from the cuticle and does not in any degree pass over into it'. He therefore regards the adhesive as due to modification of the sheath, though he adds that 'the possibility of a secretion naturally suggests itself'. In the three species here investigated the adhesions during conjugation are probably due to an alteration in the mucilage sheath, which is probably formed as a secretion.

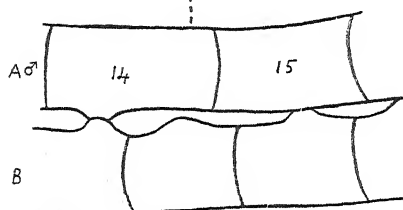
In the species to which this account refers a papilla first arises from one of two cells opposed to one another, and later a corresponding papilla is produced from the place of contact on the opposite cell. Thus, as Czurda (2, p. 449) has stated, the papillae are in contact from the first moment of their formation. By the elongation of these papillae, the pairs or bundles of filaments become pushed apart to a certain distance, which has hitherto been regarded as the original distance separating the conjugating threads. Proof of these statements is given in Diagrams I–III, Figs. 1, 2, 4, 5, 6, 8, included in this paper. The tables and figures have been selected from a large number that summarize consecutive observations on pairs or bundles of filaments, from the time they were in contact to the time that death occurred or some other misadventure befell them. In the case of *S. weberi* 26, *S. varians* 111, and *S. cataeniformis* 17 filament



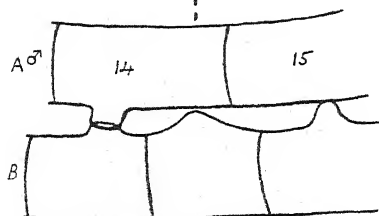
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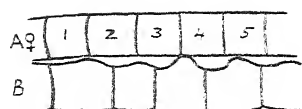


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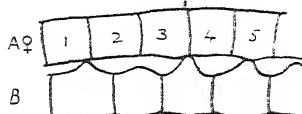


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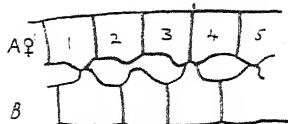
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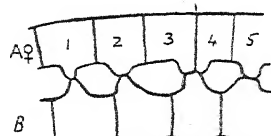
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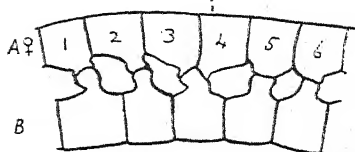
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4:0 p.m.



6:30 p.m.



11 a.m.

b

FIG. 4. Conjugation stages in *S. varians*. a and b, successive stages of conjugation in different pairs of filaments (a \times about 290; b \times about 140). See footnote on p. 236.

pairs were followed through the stages of conjugation, but the number of early stages observed was far greater.

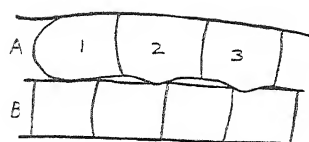
In each species search was made for evidence of the usually accepted method of conjugation, but without success in the case of *S. weberi* and *S. cataeniformis*. No filaments were observed to conjugate unless they were previously in contact and, except in cultures kept two or three days under artificial light, only two unpaired filaments with papillae were found. Even these were the projecting ends of filaments that had conjugated at other points along their length, so that they did not afford evidence for the usual view of conjugation, since the absence of the fellow filament could hardly be explained as due to loss during mounting. In *S. varians* some evidence has been obtained to indicate that *occasionally* tubes can arise and grow towards each other from cells which are *not* in contact (Fig. 6 *b*, Diagram II, *v*). This method of conjugation has only been observed, however, in portions of filaments that have conjugated elsewhere in the way described above and, after conjugation is complete, such portions can easily be recognized by the greater length of the tubes (cf. Fig. 6 *b, c, d*). This method is therefore abnormal, occurring only in those parts of a filament pair which for some reason have not become glued together like the rest. Moreover, when the main mass of the material is in conjugation, the production of abnormal papillae from the cells of unpaired filaments or portions of filaments is very frequent.

In *S. varians* in addition to those filament pairs which are obviously in contact, there are others which appear to be slightly separated, so that the small papillae on the one filament do not reach the visible limit of the other (Fig. 4 *b*). This separation is, however, not real, for the filaments cannot be moved apart and treatment of several such pairs with indian ink has shown that the space between them is occupied by a mucilage (Fig. 3 *d*).

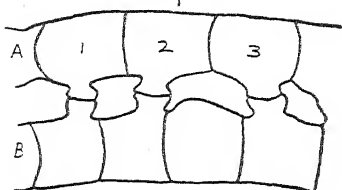
V. PAPILLA FORMATION.

It is generally assumed that it is the cells of the male filament that first put out conjugation processes. Czurda (2, p. 449) states that in species of *Spirogyra* generally, the papillae are first produced by the male cells, while, with reference to a number of species, including *S. weberi* and *S. varians*, Lloyd (9, p. 84) remarks that 'the tubes grow out in sequence, and it seems certain that the male is always the first and the female tube is consequently always the shorter'.

In the species investigated in this paper the cells which put out the first papillae are usually restricted to one filament of a pair, but this may be *either* the male filament (Figs. 5 *c*, 8 *b, c*; Diagrams II (11, iv), 3 (iv, v) *or* the female filament (Figs. 4 *a*, 8 *d*; Diagrams I (i), II (i), III (ii)). In all three species, however, a few cases have been observed in which the first

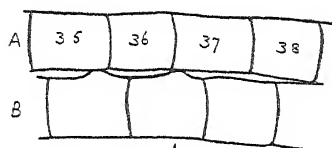


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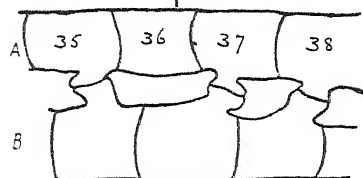


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a

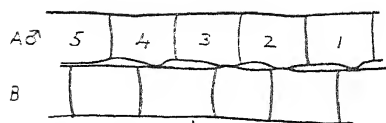


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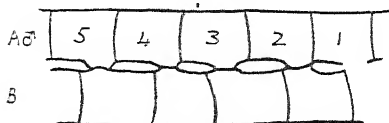


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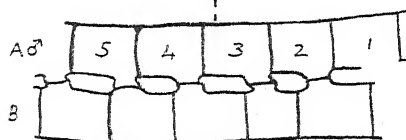
b



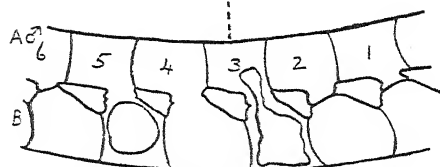
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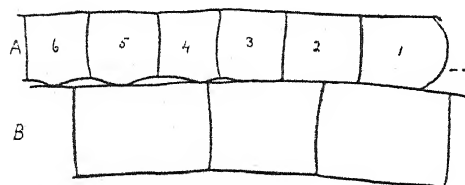


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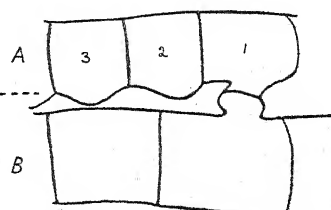


9:30 a.m. 3.6.29

c



8:20 p.m.



8:0 a.m.

d

FIG. 5. Conjugation stages in *S. varians*. a and b, successive stages of conjugation in different portions of the same pair of filaments. c and d, successive stages in different pairs of filaments. (a, b, d \times about 200; c \times about 150). See footnote on p. 236.

papillae arose indifferently from the cells of either filament (Figs. 1 *b*, 5 *a*, *b*; Diagrams I (iii, iv), II (iii), III (i, iii). Thus, although papilla formation is not simultaneous, it is not the sex of the filaments that determines which shall be first formed. No instances of cross conjugation have been observed in the three species examined, and the sex of the filaments—as shown later by the form of the cells or the position of the zygotes—is apparently determined before conjugation sets in. In *S. weberi* and *S. varians* the first formed papillae are usually very small when their fellows are produced, but in *S. cataeniformis* the papillae on the one thread sometimes grow almost to their full length, before the opposite ones develop (Fig. 8 *a*, *b*).

These results are not in agreement with those of Czurda (2), and Lloyd (9), but they conform with Haberlandt's (5, p. 393) observations, in which, however, the beginnings of papilla formation were merely deduced from the respective lengths of the male and female portions of the conjugation canals on the assumption that these grow equally in length in a given time. This, however, is not warranted, since in *S. varians* the conjugation tube has often been found to be longer on the side of the female cell in cases where the male has been observed to produce the first papilla. Again, in *S. weberi* and *S. cataeniformis* the component parts of the conjugation canals are not of unequal length, although papilla formation commences earlier in one of the two conjugating cells.

VI. RATE OF FORMATION OF THE PAPILLAE AND OF THE CONJUGATION TUBE.

In *S. varians* both formation and growth of papillae are considerably more rapid than in the other two species. Filament pairs, which were in very close contact or showed incipient papillae in the late evening (9–12 p.m.), exhibited well-developed tubes before noon of the following day. In material kept on slides under the microscope less than three hours frequently suffices for a filament pair, showing very slight papillae on one thread to advance to the stage of considerably longer papillae on both, while within twenty-four hours one thread may have differentiated swollen female cells. Slight papillae may develop within an hour and a quarter from filaments in contact with one another. Observation of material in its natural habitat shows that, here too, tube formation occurs very rapidly. The production of papillae occurs at any time of the day, so that early stages are not confined to the evening hours, as they are in *S. cataeniformis* according to Czurda (2, p. 449).

In *S. weberi* the process is on the whole much slower. In cultures under artificial light the time taken for the production of slight papillae from filaments in contact ranges from four to nineteen hours, so that filaments may lie in contact for some hours before papillae are formed. The shortest interval between the observation of filaments in contact and filaments

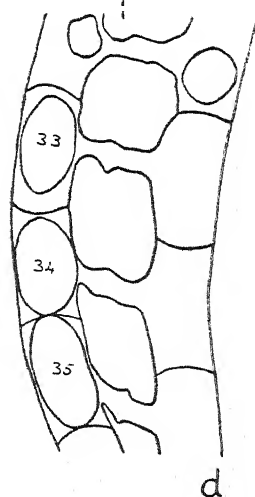
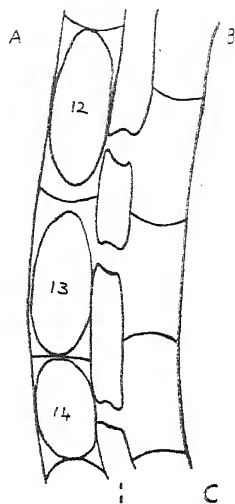
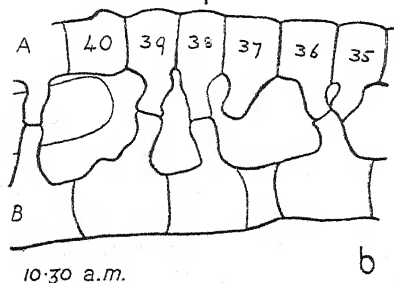
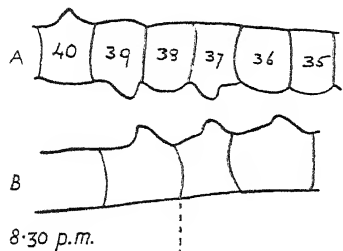
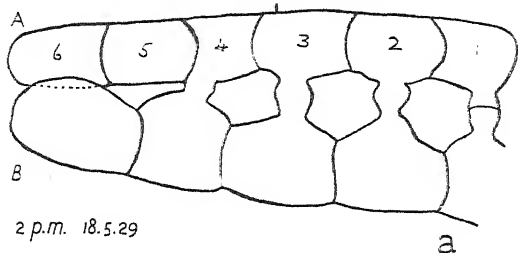
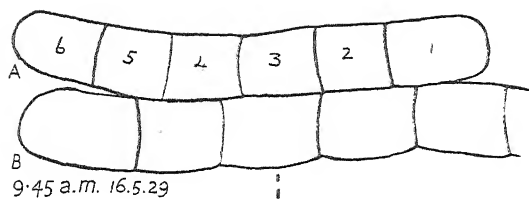


FIG. 6. Conjugation stages in *S. varians*. *a*, *b*, successive stages in different pairs of filaments. *c*, *d*, different portions of same pair of filaments. The filaments were originally in contact in the portion shown in *c*, but not in the portion shown in *d* (all \times about 200). See footnote on p. 236.

separated by conjugation tubes and showing differentiation of swollen female cells was twenty-six and a half hours; the longest was sixty-four hours. No indication of the time taken for these processes in nature has been obtained.

In *S. cataeniformis*, if and when production and further growth of papillae occur in cultures under artificial light, the process is very slow. The time taken for the production of papillae from filaments lying in contact ranges from nine to twenty-three hours and, for the formation of tubes, from thirty-six to seventy-four hours.

VII. THE POSITION OF THE NUCLEUS IN CONJUGATING CELLS.

Lloyd (9, p. 11) finds that, during conjugation in *S. crassa*, the nucleus moves from the centre of the cell towards the inner surface of the tube, although he does not regard this as lending support to Haberlandt's view that the nucleus lies at the point of most intensive growth (cf. also Czurda (2), p. 459). Czurda (2, p. 458) had previously reported that, during the formation of papillae, the nucleus without exception, shifts to the side of the cell *opposite* to that on which the papilla forms, and that in spite of exhaustive search the nucleus was never found in the neighbourhood of the papilla. He suggested, however, that in narrow species, such as *S. weberi* and *S. varians*, the nucleus might be drawn into the papilla entangled in the turns of the chloroplast.

In the present investigation the position of the nucleus has only been recorded in *S. varians*, since in the two narrower species, it could not be seen in fresh material. Whenever discernible, the nucleus in *S. varians* was situated in the middle of the cell as long as the papillae were small, and either in the same position, or on the side opposite to the papillae when these had lengthened into tubes. This evidence was supported by continuous observation of material in cultures for, during the formation of the tube, the nucleus passed from the middle of the cell to the side opposite the tube (Fig. 7 a). In short-celled filaments the nucleus could not be observed without staining, since it was hidden by the compact chloroplasts. Piconigrosin and dilute iodine, which do not alter the position of the nucleus in cells where it can be observed in the living state and cause scarcely any shrinkage, have been found useful in such cases. Such stained filaments showed that, when the tubes were fully grown the nucleus usually lay at the side of the cell which was opposite to the tube, but that when the chloroplast had grown into the tube, the nucleus was also frequently to be found there. It was never observed in or near the region of the tube unless buried in the chloroplast, and thus, as Czurda suggested, it was probably carried there passively with the chloroplast (Fig. 7 b).

VIII. SIZE AND GROWTH OF CONJUGATING CELLS.

Lloyd (9, p. 80) states that, in *S. weberi*, the male gamete is, with very few exceptions, smaller than the female, and adds that 'the exceptions show that the smaller size of the male is not essential to successful conjugation'.

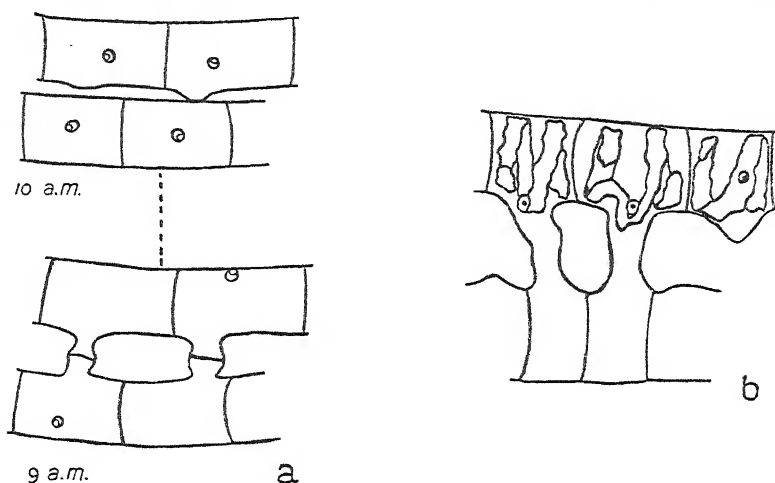


FIG. 7. Semi-diagrammatic sketches of conjugating filaments of *S. varians*. *a*, successive stages, showing the position of nuclei. *b*, filament pair stained with picronigrosin showing position of nuclei (*a* \times about 200; *b* \times about 315).

tion'. The species here investigated have not been found to conform to this rule. Measurement of the pairs of conjugating cells has shown that the cells of the male filament may be either longer or shorter than those of the female, so that length of cell is not a sexual character. The following are lengths (in microns) of corresponding cells of the female and male filaments:

<i>S. weberi</i> .		<i>S. varians</i> .		<i>S. cataeniformis</i> .	
♀	♂	♀	♂	♀	♂
76.1	47.4	38.0	38.0	37.1	35.6
64.0	76.1	47.4	58.0	35.6	40.5
59.5	76.1	38.0	38.0	40.5	35.6
56.0	69.0	35.6	40.5	43.0	33.3
Female cells longer or shorter.		Female cells longer, shorter, or equal.		Female cells longer or shorter.	

In *S. weberi* and *S. varians* this variability in the relative lengths of the male and female cells was also shown by examination of zygote material. Unequal length of the conjugating cells resulted in the production of odd, i. e. unpaired, cells in one or other filament, or in both. If the male cells were consistently shorter than the female, odd cells would be restricted to the male filament.

Both growth in length and division of the cells may occur after approximation of the filaments in conjugation, but they are not restricted to the filaments of one sex. In all three species division has been noted in the male filament only, in the female only, and in both filaments of a pair. Similarly, if growth occurs during conjugation, it may be the greater in either the male or the female cells. In the following table, which relates to *S. weberi* and *S. varians*, the length of cell was in each case measured (1) when the two filaments were merely in contact, and (2) when they had become separated by conjugation tubes. Fourteen hours elapsed between the two sets of measurements in the case of *S. weberi* and thirteen in the case of *S. varians*.

<i>S. weberi</i> .				<i>S. varians</i> .			
♀	♀	♂	♂	♀	♀	♂	♂
(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
73.6	83.0	118.0	118.0	50.0	50.0	45.2	47.4
73.6	78.0	69.0	71.3	38.0	38.0	47.4	47.4
64.0	73.6	61.8	61.8	35.2	40.5	47.4	50.0
71.3	73.6	61.8	66.0	40.5	43.0	43.0	47.4
64.0	64.0	71.3	73.6				

The marked twisting of the female filament round the male, recorded by Czurda (2, p. 446) in *S. setiformis*, and due to the greater growth of the female cells during conjugation, was not observed in my material. The conjugation tubes were usually orientated at right angles to the threads without much distortion, since growth in length of the cells was not greater in one filament of the pair.

IX. FEMALE CELLS AND ZYGOTES.

In all three species, after the early stages of conjugation had been passed through, there was a visible differentiation of sex in the shape of a swelling of the female cells, although in *S. varians* this may be very slight, and occur only on the side towards the conjugation tube. In *S. weberi* and *S. varians* female cells were never differentiated until after tube formation, the shortest interval after contact of the filament being twenty-six hours in *S. weberi* and twenty-two in *S. varians*. In *S. cataeniformis* female cells were sometimes differentiated before the fusion of the papillae, although the shortest interval was thirty-nine hours after the filaments were in contact. Zygotes were never formed in the cultures of *S. cataeniformis*, although occasionally they were in those of the other two species, but the cultures had usually become stale by the time that protoplasmic fusion occurred.

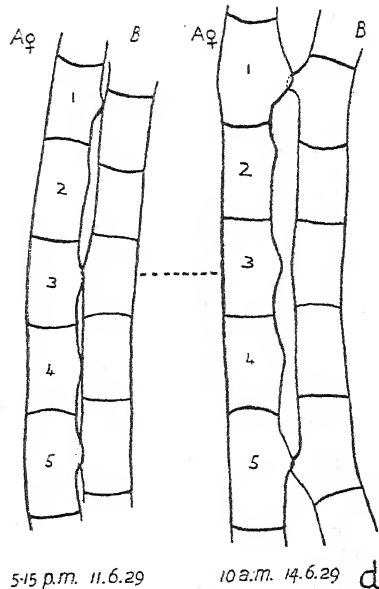
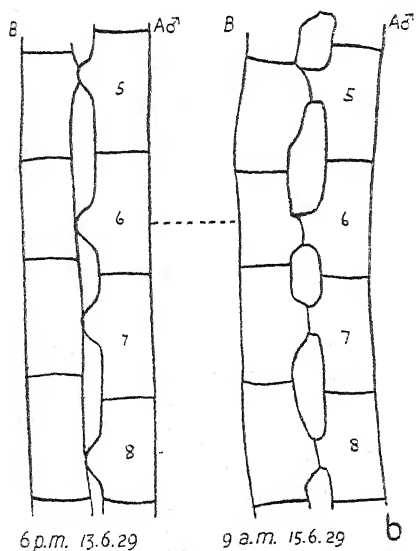
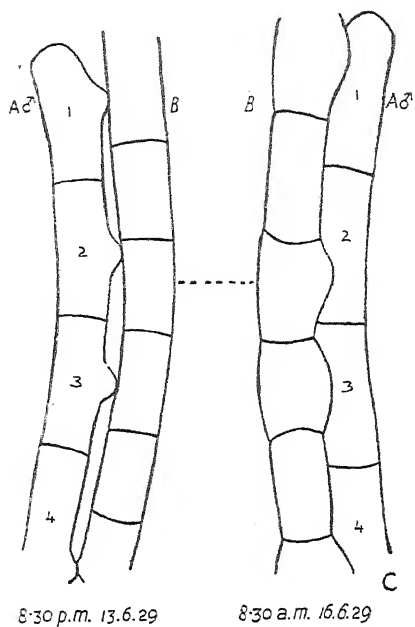
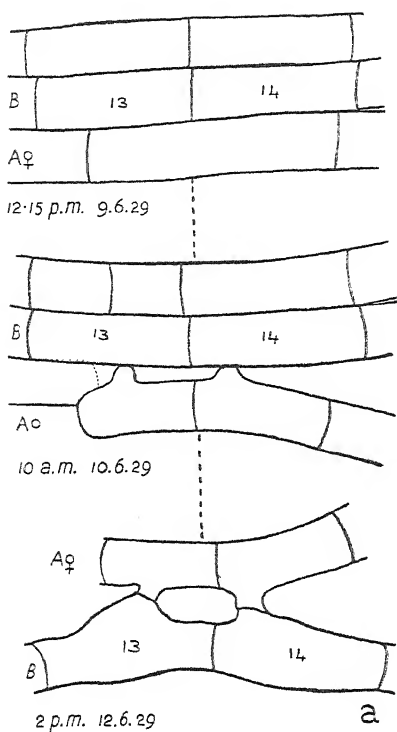


FIG. 8. Conjugation stages in *S. cateniformis*.
of filaments (all \times about 315). a, b, c, d, successive stages in different pairs
See footnote on p. 236.

Key to the Symbols used in Diagrams 1-3.

A and B are the two filaments of a conjugating pair, A being either the male or the female filament; the sex is indicated, if known by later differentiation. The size of the papilla varies, and is not in any way indicated by the symbol. Where there are no symbols it may be assumed that observations have been rendered impossible by the overlapping of the filaments.



Filaments closely in contact (mucilage sheath very thin).



Filaments in contact through mucilage (mucilage sheath wide).



Filaments separated.



Papilla from cell of filament A closely in contact with filament B.



Papilla from cell of filament B closely in contact with filament A.



Papilla from cell of filament A in contact with B through mucilage.



Papilla from cell of filament A separated from filament B.



Opposite papillae in contact.



Odd cell in filament A with no partner cell in B.



Odd cell in filament B with no partner cell in A.



Papillae flattened on each other forming a conjugation tube though the common wall is not necessarily dissolved.

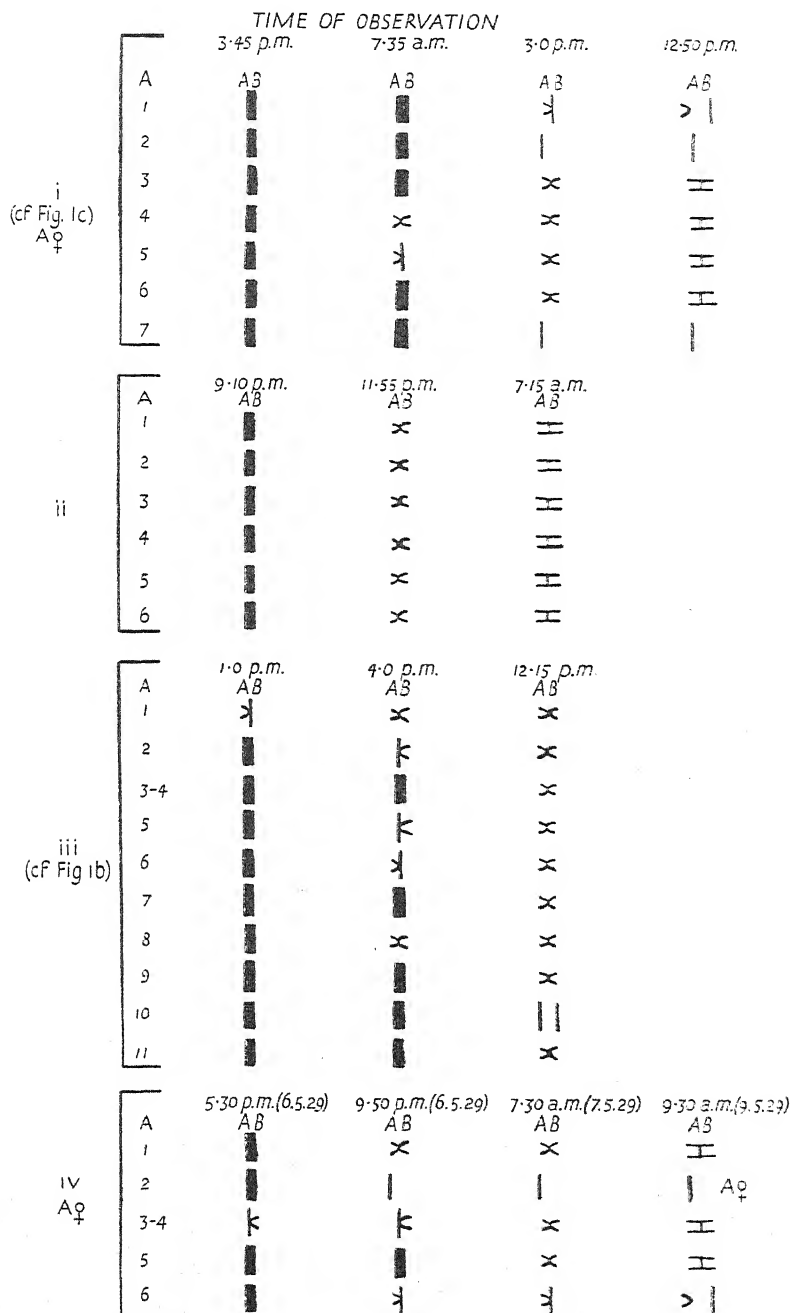


DIAGRAM I. Successive stages of conjugation in filament pairs of *S. weberi*.

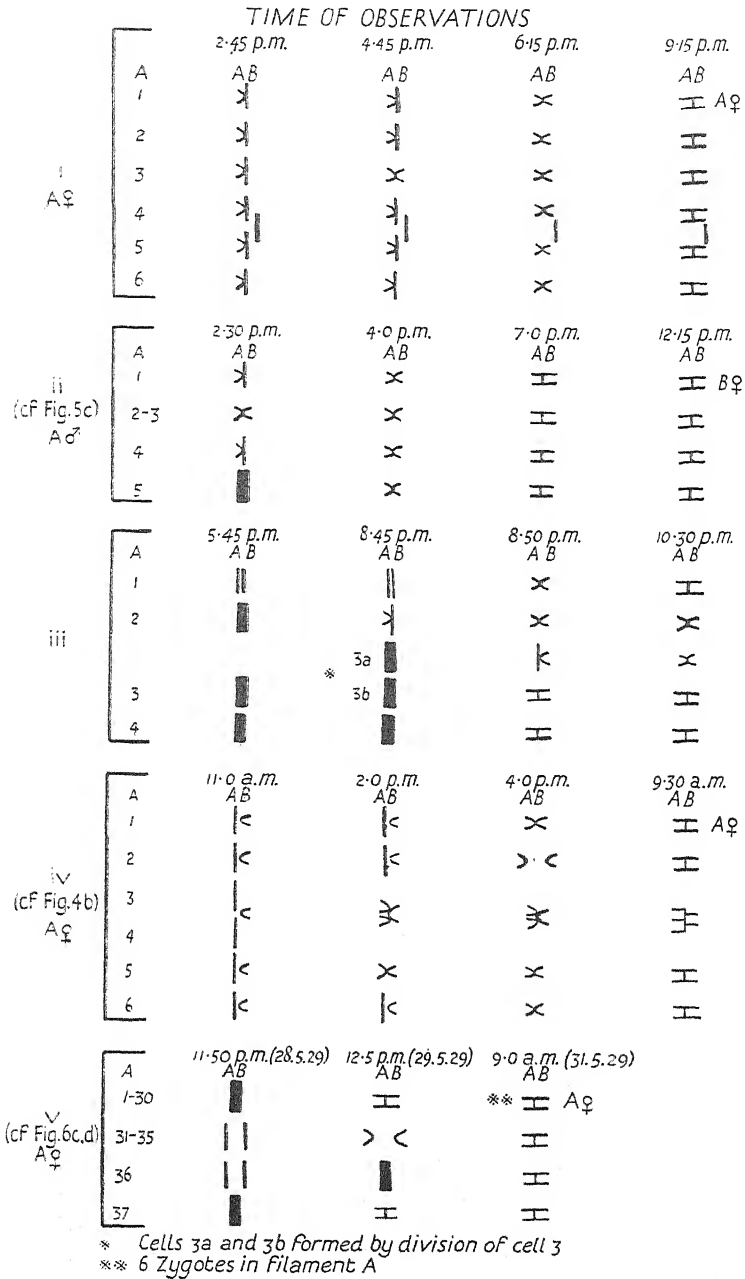


DIAGRAM II. Successive stages of conjugation in filament pairs of *S. varians*. * Cells 3a, 3b formed by division of cell 3. ** Six zygotes in filament A.

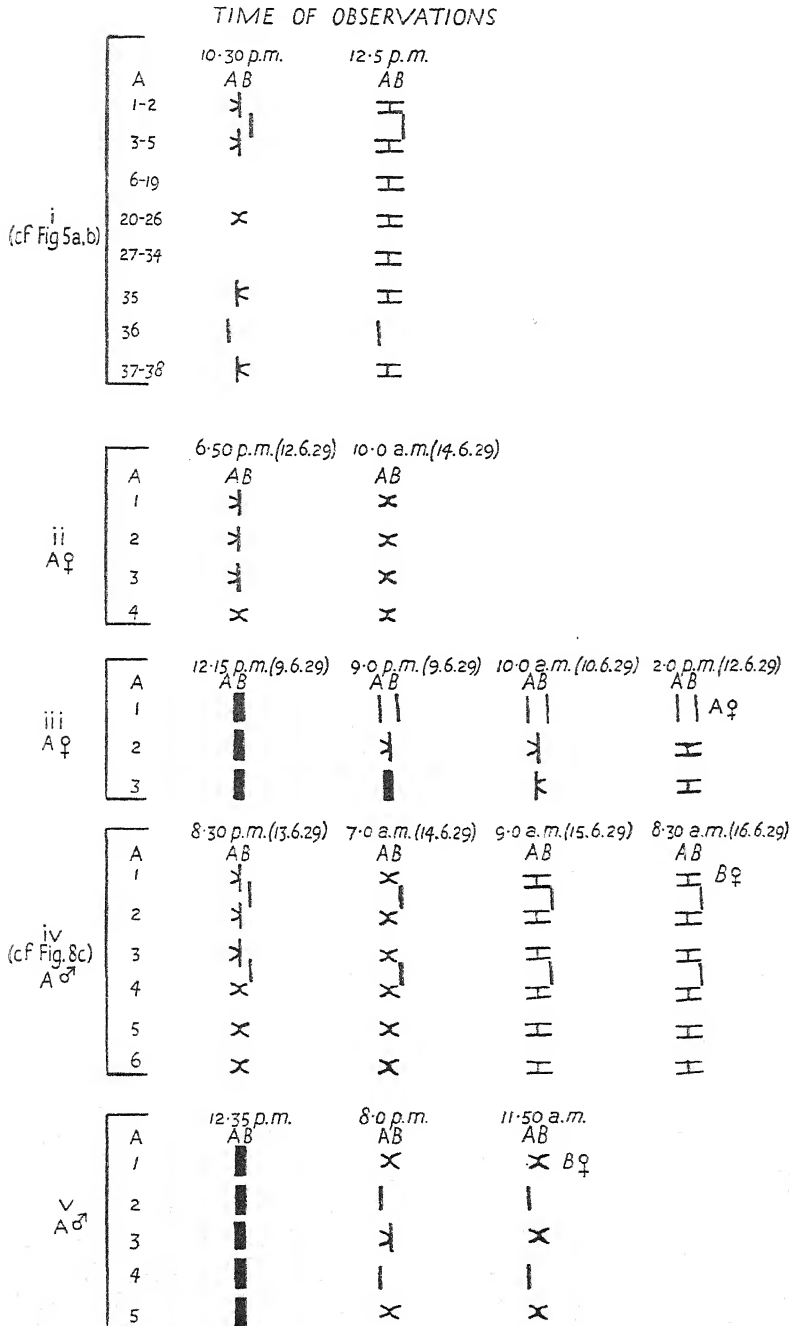


DIAGRAM III. Successive stages of conjugation in filament pairs of *S. varians* (i) and *S. cataeniformis* (ii-v).

X. CONCLUSION.

The present investigation has shown that, in *Spirogyra weberi*, *S. varians*, and *S. cataeniformis*, the generally accepted view of the method of conjugation is incorrect. This has already been reported by Czurda for a number of species, including *S. weberi* and *S. varians*, and it is therefore probable that tube formation takes place according to the method here described in all species of the genus. Hemleben (6) was the first to question the correctness of the older account; he worked on *S. crassa*, a species which was evidently used by de Bary, for the latter (3) refers to the difficulty of growing it in cultures for any length of time. In his experimental work on conjugation Klebs (7, p. 229) used *S. weberi*, *S. varians*, and other species, but he clearly did not observe early stages similar to those described by Czurda and the present writer. Lloyd (9), in his study of adhesions and geniculations, investigated *S. varians* among other species, and states (p. 108) that 'during conjugation no adhesions were observed (except, of course, of the conjugation tube)'. Both Czurda and the author, however, find that adhesions play a prominent part in the early stages of conjugation in *S. varians*.

There must obviously be some explanation why, in such a widely distributed and much used plant as *Spirogyra*, the exact method of conjugation has so long remained undetected, and a few of the probable reasons may be mentioned. Early stages are not initiated after removal of material from its natural habitat, and in nature they appear to be passed through comparatively quickly. Thus, unless freshly collected living material is examined, the early stages must of necessity be deduced from the later ones, or from the study of fixed material. Deductions from the later stages may, however, give a mistaken impression. For instance, in filament pairs laced together by conjugation tubes, the presence on opposite cells of papillae which apparently have not yet met, would suggest formation of the tubes by the meeting of outgrowths from corresponding cells. In following through slide cultures of *S. varians*, however, it has been ascertained that such unjoined papillae are at first in contact, but have been separated by the more rapid growth of the papillae from the neighbouring cells.

Since early stages of conjugation are destroyed by all the well-known fixatives (cf. p. 2), preserved material tends to give evidence in support of the method of conjugation hitherto adopted. After fixation, when pairs of filaments in early stages of conjugation have become separated, they appear as *single* filaments bearing small papillae, and would give support to the generally accepted view.

For these reasons observations on the early stages of conjugation must

be made directly on the living material. Czurda (2) undertook such investigations on the spot, but it has proved possible to carry them through in the laboratory, immediately after collection, by using special methods of culture (see p. 236). The papers of earlier workers give no indication that such methods were employed, and, as Czurda (2, p. 441) has pointed out, the older descriptions of conjugation refer almost entirely to the later stages of this process: viz. to protoplasmic fusion and zygote formation. In view of these facts, and of the great difficulty of mounting parallel filaments without displacement, it seems probable that the observations of early workers on the beginnings of conjugation were merely deduced from the later stages or from the examination of fixed material.

SUMMARY.

1. The paper deals with the conjugation of *Spirogyra* with particular reference to the changes that take place prior to the formation of conjugation tubes.

2. Observations are recorded on the early stages of conjugation in the three species *S. weberi*, *S. varians*, and *S. cataeniformis*, and are in general confirmatory of Czurda's conclusions.

3. The earliest stages of conjugation are only to be found in freshly collected material, but it has been found possible to follow their development in the laboratory.

4. In all three species the conjugating filaments at first lie in contact glued together by mucilage.

5. Papillae are later put out from one of each of the pairs of opposite (partner) cells that are glued together.

6. The first-formed papilla may arise in either filament, and is independent of the sex of the latter. The opposite papilla arises at the place of contact with that first formed.

7. The two papillae are in contact from the first moment of their formation and by their growth in length the filaments are gradually pushed apart.

8. The apices of the papillae become flattened and dissolved, and the familiar ladder-like appearance of the interlaced filaments is obtained.

9. During the early stages of conjugation in *S. varians* the nucleus moves from the centre of the cells to the side away from the tube.

10. The sex of the filaments cannot be ascertained in the early stages of conjugation, for in any filament pair the male cells may be longer, shorter, or equal in length to their partner female cells. In the later stages of conjugation there is a differentiation of sex, for the female cells become swollen.

I should like to take this opportunity of thanking Professor F. E. Fritsch for suggesting this research, and helping in its preparation for publication, and Miss E. M. Blackwell, for her continued interest, encouragement, and criticism, during the investigation.

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Two New Species of *Pythium* Parasitic in Green Algae.¹

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With Plate IX and two Figures in the Text.

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INTRODUCTION.

SINCE the publication of Butler's epochal monograph of the genus over twenty years ago (6), but few instances of species of *Pythium* parasitic in algae have been reported in the literature. It has seemed desirable, therefore, to undertake a detailed study of certain species occupying this type of habitat, not only for the reason of their apparent rarity, but also because phases in their morphology and life-history seem little understood. Two species of *Pythium*, both of which were found parasitic in algae, have been investigated in detail by the writer and the results of this study are embodied in the following paper.

MATERIALS AND METHODS.

The two fungi were found parasitic in green algae collected in the vicinity of Cambridge, Massachusetts. One, occurring in filaments of *Rhizoclonium hieroglyphicum* (Ag.), Kützing, collected in a small pond in Fresh Pond, Parkway in October, 1926, appeared in March, 1927, in a

¹ Contribution from the Cryptogamic Laboratories at Harvard University no. 105.

laboratory culture of this alga, proved to be a new species, and is herein designated as *Pythium adhaerens*, n. sp.; the second, which was found in filaments of *Spirogyra crassa*, Kützing, collected in the aforementioned pond in May, 1928, also proved to be a new species and will be termed in this paper, *P. angustatum*, n. sp.

In isolating the two fungi the following method was employed. Infected filaments of the alga were thoroughly washed in sterile water, transferred to a sterile slide, and examined microscopically for free zoospores and for indications of any fungus other than the desired one. Oospores and attendant mycelium were separated from the algal filament, and were planted in petri dishes of nutrient agar, where colonies were readily produced. Bacteria-free cultures were obtained by a process of repeated transference from the advancing edges of the colonies. The identity of the two fungi was obtained by comparison with those in the algal filaments.

Non-sexual reproduction was obtained in pure culture by washing colonies of the fungus, grown in nutrient solutions, in sterile distilled water. Zoospores were produced in week-old colonies by this method in 10–15 hours. Sexual reproduction occurred in great abundance in cornmeal, oat-meal, carrot, potato-dextrose, and maize-oil agars, the latter prepared according to the method of Kanouse and Humphrey (14). Only a luxuriant vegetative growth developed on castor-oil agar.

Single spore cultures were made according to the method advocated by Kauffman (15).

MORPHOLOGY AND DEVELOPMENT OF THE FUNGI.

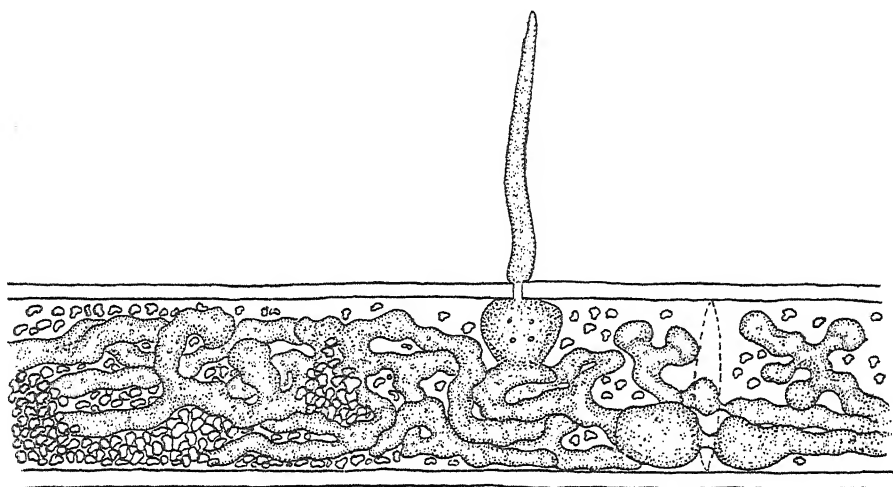
(a) *Pythium adhaerens*, n. sp.

The macroscopic indication of the presence of the fungus was the appearance of several small, pale brown areas on the otherwise dark green mat of *Rhizoclonium*. The filaments were found to be heavily parasitized by the coarse, coenocytic mycelium of this fungus (Text-fig. 1). The extramatrical mycelium exhibited numerous pyriform appressoria, which had apparently developed wherever the tips of the hyphae came into contact with the wall of the host. These discoloured areas soon increased enormously in size, and others appeared in widely separated locations, the fungus soon establishing itself within nearly all of the threads in the upper region of the mat.

Mycelium. The intramatrical mycelium is greatly distorted. The main filaments and branches, which are from 5 to 7.2 μ in diameter, arise at frequent intervals and are closely interlocked. At first the mycelium is almost wholly intramatrical, ramifying throughout the entire filament. Upon penetrating a wall of the host, the hyphal apex becomes greatly distended and there develops on the appressed surface of the enlargement

a fine tube which pierces the wall and, on the opposite side, expands to the normal size of the hypha. Ultimately, branches of the mycelium similarly penetrate the lateral walls of the host, and form a profusely branched extramatrix growth which infects an enormous number of healthy algal filaments.

Infection of the algal cell takes place as follows: The tip of an extramatrix hypha becomes adherent to the algal wall by means of a refractive,



TEXT-FIG. 1. Habit of *Pythium adhaerens*, n. sp. within a portion of a cell of *Rhizoclonium*, showing the intramatrix appressoria. Redrawn from a photomicrograph.

mucilagenous secretion. Subsequently, the hyphal apex becomes slowly distended forming a securely anchored pyriform body. These structures, similar to those mentioned by other writers as occurring in various species of *Pythium* when grown in culture dishes, here reveal their true nature; that is, they serve as appressoria. These are usually found in clusters of two to five, or singly (Pl. IX, Fig. 2). A slender refractive tube is formed at the tip of the appressorium, and penetrates the host-wall (Pl. IX, Fig. 5). The wall of the alga in the region of the penetration tube may be markedly depressed, which suggests mechanical action (Pl. IX, Fig. 5). Often, however, no such depression is observable, and this, together with the modified nature of the highly refractive tube, seems to suggest that a hydrolytic cellulase aids penetration.

Appressoria are also formed in pure culture whenever the hyphae come into contact with the glass surface of the culture dish. These are slightly arched and clavate, or slender and somewhat sickle-shaped, occurring singly or in clumps of great complexity. Figures 3 and 4 of Pl. IX, show the hyaline concave adhesion disks. When treated with dilute solutions of gentian violet and methylene blue, these are deeply stained,

suggesting a modified cementing substance. A similar type of material has been described as occurring in the appressoria of *Botrytis cinerea*, Pers., by Blackman and Welsford (3). The stimulus which induces the formation of these appressoria is apparently of a physical nature, namely, contact. Dissmann (9) has found that similar bodies are formed in *P. proliferum*, de Bary, in Petri dishes of media whenever the hyphae come into contact with the surface of the glass. He believes that the alkalinity of the glass is the important factor and states that appressoria were obtained in liquid media when a mat of mycelium, grown in a solution of haemoglobin, was washed and placed in distilled water at pH 8.4. No appressoria were produced by Dissmann's method in the case of the fungi described in this paper.

Non-sexual Reproduction. Coincident with the development of the extramatrical mycelium, the formation of sporangia and zoospores was frequently observed in the algal culture.

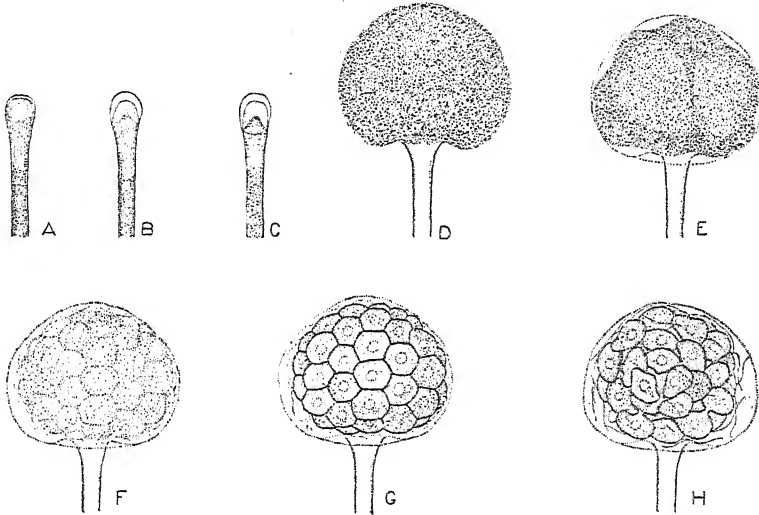
The zoosporangia are unbranched or, more often, branched portions of the extramatrical mycelium, which extend for varying distances out into the water. Quite often globose to sub-globose intramatrical appressoria occur in more or less compact intercommunicating clumps, connected with the hypha which has effected penetration of the host-wall (Pl. IX, Fig. 1). This hypha functions as an evacuation tube, discharging not only its own content, but that of the complex of intramatrical appressoria and their concomitant hyphae. In pure culture, no such swellings were ever found, indicating that their formation was the result of their growth in the *Rhizoclonium*.

Whether observed in the alga or under conditions of pure culture, certain constant changes take place in the vegetative mycelium preceding zoospore formation. Numerous vacuoles appear in the densely granular content of the mycelium, but after several hours decrease in size, and finally disappear. In the now finely granular protoplasm, narrow cross walls may be observed laid down at considerable intervals along the mycelium. These blocked-off hyphal portions are the initials of the zoosporangia. Following this, the tips of certain hyphae (one for each continuous mycelial segment) show striking modifications which indicate that from them the evacuation of the content will take place.

Within the apical curvature of these hyphal tips a narrow zone of hyaline, highly refractive material develops, which appears in optical section as a slender crescent (Text-fig. 2, A). This substance is apparently derived from the apical wall. There may be detected, in surface view, at the same time and often before the refractive dome becomes visible, a vacuole immediately below the hyphal tip (Text-fig. 2, A), which increases in size and perceptibility with the enlargement of the apical material. The dome of glistening substance appears to possess, shortly before the dis-

charge of the content, a double contour when viewed in optical section (Text-fig. 2, B, C). The evacuation of the protoplasm is preceded by the same rapidly occurring changes which transpired in *P. dictyosporum*, Racib., where they are described in detail by the writer (22).

With the expansion of the apical refractive material, protoplasmic ejection is initiated. The dilatation of the refractive dome keeps pace with



TEXT-FIG. 2. Formation of zoospores. From camera lucida drawings of living material. $\times 622$. A, B, C. Stages in the modification of the tip of the evacuation tube (optical section). D. Protoplasm immediately after emergence. E. Irregular clumping of the protoplasm; vesicle now visible. F. Formation of cleavage furrows and first appearance of cilia. G. Appearance of vacuoles in spore initials. H. Maturation of zoospores nearly completed.

the effluent protoplasm, and forms around it an extremely tenuous vesicle. This structure is never exactly spherical because the somewhat flaring tip of the evacuation tube protrudes slightly into its lower portion (Text-fig. 2, D).

The ejected protoplasm appears as a smoothly granular, homogeneous mass which completely fills the vesicle (Text-fig. 2, D). Immediately after discharge a slight surging movement of the minute particles of the protoplasm is perceptible. After a few seconds slight contractions in certain regions (Text-fig. 2, E) lend a lumpy, irregular appearance to the mass. Faint peripheral lines of cleavage, which demarcate irregularly polygonal areas, soon become visible in the now slightly more contracted content.

About three minutes after discharge a rocking movement of the mass is noticeable. The lines of cleavage have by this time definitely delimited the spore initials (Text-fig. 2, F), which approximately six minutes after the discharge exhibit a slight individual movement. Short, hyaline cilia may now be definitely seen around the periphery. The movement of the

spore initials gradually becomes more pronounced, assuming a twisting or writhing character as they slowly become separated from each other. At about eight minutes a small vacuole becomes visible in each of the spore masses, and the previously short, hyaline cilia have increased in length, and appear as dark, flexible lashes (Text-fig. 2, G). Three minutes later, the spores have become nearly mature individuals. They still continue, however, to oscillate somewhat until they are perfectly formed (Text-fig. 2, H). About fourteen minutes after egress this motion is gradually superseded by a frenzied milling around of the zoospores within the confines of the vesicle. Ultimately, the vesicle is ruptured by one or more zoospores, usually in the upper portion, and the immured swarmers escape. The vesicle is ultimately dissolved into the surrounding medium.

Variations from the foregoing typical instance of zoospore formation were no greater when the two species described in this paper were contrasted than they were when different sporangia of the same fungus were compared, and are probably due to differences in the environmental conditions. No evidence obtained from observations on protoplasm migrating into the vesicle, on whole mounts of killed and stained undischarged sporangia, or on sectioned and stained material, indicated that the zoospores were formed prior to sporangial discharge.

Previous investigators have made various conjectures as to the nature of the substance making up the refractive dome from which the vesicle is derived. De Bary (2) termed it a 'gelatinously thickened wall', while Ward (24) and Butler (6) considered it to be probably of pure cellulose or a modification of this substance. In microchemical tests designed by various investigators to indicate the presence of cellulose, hemi-cellulose, callose, protein, and pectin materials, positive results were obtained only with those which have been emphasized as specific for the last-named substance. The dome is also stained slightly by muchameatein, a mucilage stain. It was noticed that when the dome was stained, the dye coloured even more deeply the protoplasm immediately below this structure. This suggests that the content in this region of the sporangium is modified for the production of the vesicular material.

The zoospore of this species, in general, resembles the secondary, laterally biciliate type figured and described by Weston (25) for *Thraustotheca*, one of the Saprolegniaceae, and by Arens (1) for *Pseudoperonospora*, a member of the Peronosporaceae. However, it exhibits certain morphological characters that mark it off as distinct. Chiefly, these are the more pointed, somewhat upturned ends and the slightly higher sides in the mid-region, points hard to express but easily seen in the drawings (Pl. X, Figs. 6, 7, 8). A nucleus to which the cilia seem to be attached is found in the mid-region. The zoospore is generally $10.8\ \mu$ long by $6.4\ \mu$ at its greatest width. It moves in a helicoid path through water (21°C.) at a lively rate

for three to five hours. The rate of movement is noticeably slower at low temperatures. When coming to rest, the spore assumes an ovoid, and then a spherical shape, and the cilia seem to be slowly contracted into the body (Pl. IX, Figs. 9 and 10). The cystospore thus formed varies from 5.4 to $9.0\ \mu$ in diameter, generally averaging $7.2\ \mu$. Penetration of the host cell-wall is accomplished in the same manner as that described for *P. dictyosporum*, Racib., by the writer (22).

Most of the cystospores produce a narrow germ-tube which, under favourable conditions, soon enlarges to a hypha of typical diameter. In Pl. IX, Fig. 11, a small appressorium is shown which has been formed at the tip of the hypha. In distilled water, oftentimes the cystospore undergoes a quite different type of development, termed 'repeated emergence', in the sense of Weston (26). This process, similar to the zoospore formation previously described, results in the production of a single zoospore, similar in shape to its progenitor, but slightly smaller ($9.0\ \mu$ by $5.4\ \mu$). A vesicle may or may not be formed (Pl. IX, Figs. 12 and 13).

Sexual Reproduction. Sexual reproduction began to develop within occasionally heavily parasitized cells of *Rhizoclonium* about ten days after the fungus appeared in the algal culture. Due to the complex of vegetative elements and sexual organs, the exact relationships of the latter were determined with difficulty.

In pure culture, on a wide variety of media, no sexual organs were produced when the fungus was first isolated. After two months, oospores were noticed in one tube of nearly dry oatmeal agar. This culture was one of those obtained from the first colony of the fungus which appeared as a result of inoculation with a bit of mycelium from the infected *Rhizoclonium*. Subsequent transfers from this oospore-bearing strain have never failed to produce an abundance of sexual bodies in culture which agree in every detail with those formed in the alga. The other strains, isolated at the same time and grown under similar conditions, have continued to remain sterile after three years in culture. Aside from the absence of sexuality, the vegetative and non-sexual reproductive structures of the sterile forms cannot be distinguished from those of the fertile. Such an instance closely parallels those described by Braun (4) for *P. debaryanum* var. *Pelargonii*, Braun, and Matthews (16) for *P. papillatum*, Matthews. In addition, Kanouse and Humphrey (14) were unable to induce sexual reproduction in *P. afertile*, K. and H., although the fungus was grown on various culture media.

In pure culture on clear cornmeal (maize) agar, the details of the formation and the relationships of the sexual organs were observed with great clarity. The oogonium originates as a terminal or intercalary swelling of the hypha, which gradually increases in size until it is, when finally mature, a spherical body, $11-26\ \mu$ in diameter, cut off by cross walls from

the adjacent hyphae. The antheridia are diclinous in origin and arise as short, lateral, expanded hyphal branches, which clasp the oogonium early in its formation (Pl. IX, Fig. 14). Two to four male organs are generally in contact with an oogonium, the single hypha from which the several antheridia usually arise often surrounding the female structure in a characteristic manner (Pl. IX, Fig. 15). When fully mature each antheridium is delimited from the hypha by a cross wall, formed generally about 15μ from its tip which is in contact with the oogonial wall. Fertilization is accomplished in three to five hours by the gradual transference of all, or nearly all, of the antheridial content to the oogonium through a cylindrical, refractive tube about 2μ in diameter, similar to the appressorial penetration tube, which penetrates the wall of the oogonium and extends an unknown distance into the ooplasm.

Coincident with the formation of the fertilization tube, the content of the oogonium becomes more condensed and contracts away from the wall. The ooplasm, connected by hyaline strands of protoplasm to the oogonial wall, generally lies in an eccentric position, in close contact with the region of the wall penetrated by the fertilization tube. During fertilization the content, instead of possessing a smooth, densely granular consistency, becomes darker and extremely irregular in contour. As the discharge of antheridial material progresses, the ooplasm continues to contract and the fine droplets of oil which are distributed throughout combine to form large, irregularly shaped, highly refractive bodies, which finally compose nearly the whole mass.

After fertilization, the contour of the oospore becomes more even, the refractive bodies rapidly decrease in size, and the protoplasm becomes coarsely granular. No differentiation of gonoplasm and periplasm could be observed in living material. There is now formed a thin pellicle around the ooplasm which gradually increases in thickness until it attains a breadth of $2-2.5\mu$. Finally, during the formation of the wall, the oil droplets, dispersed throughout the somewhat mottled oogonial protoplasm, unite to form a large, centrally disposed, refractive globule, ordinarily about one-third the diameter of the oospore. There is also between the inner face of the wall and the oil globule, a lenticular nucleus (Pl. IX, Fig. 16). The mature oospore lies loosely within the oogonial wall, and varies from 7.5 to 22μ in diameter, the majority being around 14.5μ . These were found to maintain their vitality after being frozen in ice for three months and after drying for a like period of time and, as mycelium and zoospores when subjected to similar conditions failed to revive, they are undoubtedly the means whereby the fungus survives adverse environmental circumstances in nature.

Germination of the Oospores. Attempts by previous investigators (Ward (24), Gobi (13), Braun (4), Edson (11), and others) to germinate the oospores in species of *Pythium* were unsuccessful. In *P. adhaerens*, n. sp.,

however, it was found possible to induce this in the following manner: petri dishes of cornmeal and oatmeal agar, containing oospores, were exposed to outdoor temperature from February to April. Pieces of the dried, coriaceous media were then scraped out and placed in capsule dishes containing sterile distilled water and left at 26° C. for twelve hours. Examination at the end of this period revealed an abundance of germinated oospores.

The stages in the germination of the oospore are not noticeably different from those of other species of *Pythium*. The large oil globule disassociates into a number of refractive parts that are finally homogeneously dispersed throughout the now evenly and finely granular protoplasm. Coincident with these changes, the oospore wall is steadily decreasing in thickness, apparently being absorbed by the protoplasm. The germ hypha, somewhat constricted as it pierces the spore-wall, elongates, branches, and establishes the new mycelium (Pl. IX, Figs. 24, 25, 26). In water, certain of its branches may be converted into sporangia. Only vegetative development takes place in nutrient solutions.

The behaviour of the swarm spores which are produced in the germination of the oospores in sterile water is exceedingly unusual, and, so far as can be determined, has never been reported as occurring in any member of the Phycomycetes. In an experiment originally designed to determine whether or not the cystosporic germ-tubes possessed a definite response to light, ten Van Tieghem cells containing zoospores from germinated oospores were placed in a black box with a slit at one end. Ten others were placed on top of the box, exposed to light on all sides, the whole being left at room temperature (21° C.). After five hours, the usual duration of the swarm period, all were examined. All but a few scattered zoospores had come to rest and germinated, but no specific response to light was observed. Upon a chance examination several hours later, it was found that all of the cells contained innumerable active zoospores, estimated as being many more than were originally present in the drop. Many empty cystospores, as well as zoospores in various stages of repeated swarming, were observed. Furthermore, it became apparent that the germinating cystospores in all cases gave rise to *two to five* zoospores (Pl. IX, Figs. 17, 18, 19). The actual process of repeated swarming, requiring only about six minutes, was entirely similar to that described under the section *Non-sexual Reproduction*, save that in the latter case only one zoospore was produced. The motile bodies thus formed were similar in shape to those previously described. They were, however, smaller in size, being usually 7.2 μ in length by 5.4 μ in width.

It now seemed desirable to determine whether or not the zoospores were capable of further repeated germination. A drop of water containing this 'second generation' of swarm spores was transferred by means of a sterile pipette from each of the afore-mentioned hanging-drop cultures and

placed in new ones. The approximate number of active zoospores and the position of any empty cystospores were noted. These were left over night at room temperature ($21^{\circ}\text{C}.$), and examined about twelve hours later (nineteen hours after the original transfer to hanging-drop cultures). In each cell it was observed that not only were there active zoospores, but that the number of empty cystospores had increased. Furthermore, in several of the cells the process of repeated swarming was observed. In view of the length of the swarm period (about five hours at $21^{\circ}\text{C}.$) and the increase in the number of empty cystospores, *at least* one repeated emergence must have occurred during the night. The process of repeated swarming observed at this time was therefore giving rise to a 'fourth generation' of zoospores. A few of the zoospores were seen to be still motile forty-eight hours, and in one case three days, after the original mounts were prepared.

The ability of each of these zoospores in the absence of available nourishment to become a miniature sporangium, and to swarm repeatedly has several far-reaching effects. Since each cystospore gives rise to *several* zoospores, the number of these bodies, each of which is capable of reproducing the organism, is enormously increased. The chances are fewer, therefore, that the fungus will fail to reach a favourable substratum. Furthermore, the period of motility, during which the zoospore is seeking a habitat favourable for growth, is greatly increased by its ability to emerge repeatedly as a motile body.

One further point of interest was observed regarding the behaviour of these zoospores. The hanging-drop cultures used in the foregoing work were left for five days at laboratory temperature ($21^{\circ}\text{C}.$). At the end of that time they were examined for any further signs of zoosporic activity. No motile zoospores were observed, but in several of the cultures small oospores were found. A number of cystospores had succeeded in producing a rudimentary mycelium of varying length. In some cases, where the hyphae of two cystospores had intermingled, there was found, on a branch of one of these, a small oogonium, about 10μ in diameter, while the mycelium from the other cystospore had produced one or two small antheridia (Pl. IX, Figs. 20 and 21). In one instance, shown in Pl. IX, Fig. 22, no antheridial structure was formed, but a short refractive tube, produced as a lateral outgrowth of the hypha, merely penetrated the oogonium, and discharged into it a portion of the hyphal protoplasm. About ten hours later it was found that a thick-walled oospore, slightly smaller than the oogonium, had been formed (Pl. IX, Fig. 23). In its proportions and general appearance it was exactly like that previously described for the species, but smaller. Although observed for over a week these oospores did not germinate. Other hanging drop cultures, similarly prepared and left under similar conditions, exhibited several cases of the formation of small sexual organs, as well as abundant

repeated swarming. As has previously been intimated, these sexual bodies were not found every time the hyphae of germinated cystospores had intermingled. This, together with the absence of androgynous antheridia, seemed to suggest that the zoospores were of but one sex. The sterile cases could then be explained as possibly being due to the intermingling of hyphae of only one sex.

Sexuality of the Fungus. From the evidence described above, it seemed desirable to ascertain whether or not the fungus was heterothallic. In April, 1927, two series of single spore cultures were made. The first was derived from zoospores produced by a mat of mycelium which had been grown in pea-bean broth at room temperature. The inoculum used was mycelium from an oospore-producing strain. Zoospores were produced after washing in sterile water at 21° C. Ten single spore cultures were obtained from this source, none of which produced oospores on various media. Eight more were then obtained from zoospores produced from oospores germinated in water. No sexual bodies were formed in any of these single spore cultures. When these strains were contrasted in all possible combinations on plates of cornmeal agar (a favourable medium for oospore production), no sexual bodies were produced. These combinations were repeated on various media, but the sterility persisted. The chances are exceedingly slight of taking up at random a drop of material containing motile bodies of only one sex if the dish contained thousands of zoospores of both sexes. That this would happen in both series is even less probable.

In September work was resumed on this point. A third series of single spore cultures was prepared from mycelium grown in corn broth, using the same inoculum as in series 'one'. The colony was washed and left at 26° C. over night. Twenty-seven single spore cultures were obtained, all of which produced oospores in the standard cornmeal agar. This indicated that leaving the washed mycelium at 26° C. had perchance caused the production of oospores. A series was therefore made up in a similar manner, but the washed mycelium was left at room temperature (21° C.). Of the forty-one monosporic cultures obtained from this lot, all produced oospores. This indicated that the change from pea-bean broth to corn broth possibly had induced oospore formation. Therefore the experiments were repeated under the conditions of those of the previous spring. Over fifty single spore cultures were thus obtained, all of which produced oospores.

In brief, the facts regarding the sexuality of this fungus may be recapitulated as follows: first, oospores were formed in but one of the several tubes inoculated with material taken from the original colony of the fungus, and then only after three months, and subsequent transfers from the oospore-bearing strain have always produced sexual organs, while the sterile strains have remained sterile; secondly, no androgynous antheridia

were ever observed; thirdly, germinated cystospores were seen, on the hyphae of which there were produced either antheridia or oogonia, never both; fourthly, single spore cultures derived from (a) mycelium from an oospore-producing culture, from (b) germinating oospores, remained sterile, as did all combinations of these strains; fifthly, five months later, with as near a duplication as possible of the conditions prevailing in the previous cases, all newly isolated single spore strains produced oospores.

The first, fourth, and fifth points would seem to indicate that the fungus was homothallic, but that sexual reproduction may be suppressed by environmental conditions. The second and third, that the fungus was possibly heterothallic.

Preliminary attempts were made to discover what external factors, if any, might be influencing the sexuality. For example, additional single spore cultures were obtained from mats of mycelium left in water, after washing, at 3° C., 10° C., 21° C., and 26° C. Again, in all cases, oospores were produced.

It is highly probable that certain factors, such as the amount of food material left in the mycelium after washing, the pH. of the water in which the mat is left, &c., vary considerably in different mats of mycelium used to obtain zoospores. The identification and segregation of these factors, as well as their control, would require an exacting physiological investigation and, as the present paper is primarily a morphological one, it has not seemed justifiable to do more than indicate at this time that the sexuality of *P. adhaerens*, n. sp., appears to be affected by certain unknown environmental conditions. The writer has in preparation a projected study of this question.

(b) *P. angustatum*, n. sp.

Filaments of *Spirogyra crassa* parasitized by this fungus could be recognized by their gray, ashen appearance, which was in contrast to the dark, rich green of healthy, uninfected threads.

Mycelium. Within the infected *Spirogyra*, this fungus formed a non-septate, irregularly branched mycelium, which spread from cell to cell, perforating the end walls, and occasionally sending out branches through the lateral host walls. These very slender, practically isodiametric hyphae possessed a finely granular content, and were 2–3.7 μ in diameter. Often a slight swelling of the hypha was formed where it penetrated the wall of the host, but this was by no means of constant occurrence. Small appressoria similar to those of *P. adhaerens*, n. sp., were occasionally seen (Pl. IX, Fig. 28).

In pure culture, the fungus grew well on a variety of media, agreeing in morphological particulars with the organism in the alga. No appressoria, however, were formed under these conditions.

Non-sexual Reproduction. Sporangia were not observed when the fungus was first examined, but when infected algal filaments were placed at a temperature of 5° C. for several hours, they were present in abundance. These structures are undifferentiated from the vegetative hyphae and, save for their more delicate nature, cannot be distinguished from those of *P. dictyosporum*, Racib., recently reported by the writer (22). When the fungus was studied, either in nature or under conditions of pure culture, the sequence and character of the changes leading to the formation of zoospores exhibited no points of difference from those described for *P. adhaerens*, n. sp., except that the vesicle was nearly invisible, and that oftentimes after the expulsion of their content the sporangia appeared to collapse (Pl. IX, Figs. 29 and 30). The vesicle was about 15 μ in diameter, and after the escape of the zoospores almost instantly deliquesced.

From two to eight zoospores are ordinarily formed in each vesicle (Pl. IX, Figs. 27 and 30), although as many as ten have sometimes been observed. These are of the laterally biciliate type, similar to those of *P. adhaerens*, and are ordinarily about 8.5 μ long by 4 μ wide (Pl. IX, Fig. 31). The period of motility, the germination of the cystospore (Pl. IX, Figs. 32, 34, 35), and the penetration of the alga are similar to those of *P. adhaerens*. The cystospore measures about 7.2 μ in diameter. Repeated swarming has also been observed to occur in the zoospores of *P. angustatum*, n. sp. (Pl. IX, Fig. 33). The shape of the swarmer is similar to the primary one, although it is slightly smaller in size. No vesicle has been observed surrounding the emerged material, although it is possible that it is present but exceedingly tenuous.

Sexual Reproduction. Sexual organs were found in abundance in heavily parasitized algal cells. They were also formed in ordinary laboratory media, such as cornmeal or oatmeal agar.

In clear cornmeal agar three days after inoculation, the oogonia originate as distended terminal or intercalary portions of the hyphae. At maturity they are spherical, subspherical, or oftentimes, sac-like bodies, cut off from the adjacent hyphae by narrow cross walls. Their diameters vary from 13–27 μ , the majority, however, measuring 20 μ .

The antheridia, which number from one to five to a single oogonium, are usually diclinous in origin (Pl. IX, Fig. 36), but may be androgynous (Pl. IX, Fig. 38). In either case, they originate as branched or unbranched, slender, somewhat tortuous hyphae, which, on coming into contact with the oogonium, expand in the distal region. The mature antheridium, which is delimited by a basal septum from the adjacent hypha, is usually slightly constricted in its mid region, and possesses a rather blunt apex, which makes broad contact with the oogonium (Pl. IX, Fig. 36). When fully mature it is usually 8.5 μ long by 5 μ wide. From each of the antheridia attached to an oogonium, a fertilization tube, 2–2.5 μ in diameter, is formed,

and through this the entire content is evacuated into the egg, after which the tube soon disintegrates (Pl. IX, Fig. 37).

The changes occurring in the ooplasm during the process of fertilization and the subsequent maturation of the oospore do not differ markedly from those previously described for *P. adhaerens*. The oospore, when mature, varies from $11-25.5\ \mu$ in diameter, averaging $18\ \mu$. This organ is characterized at once by the tenuity of its wall, which is usually $1-1.5\ \mu$ thick, and proportionately thinner in very small oospores (Pl. IX, Figs. 37, 38, 39.) Another feature is the frequency with which two or more oospores are formed within a single oogonium. The number of multi-spored oogonia may be as high as 3 per cent. of all those found in a single mount of material (Pl. IX, Fig. 40). The details of this condition and possible reasons for it await the results afforded by a cytological study.

Germination of the Oospores. Germination of the oospores occurs whenever they are placed in water or fresh nutrient material, no period of rest being necessary. This process is similar to that found in *P. adhaerens* and culminates in the production of a germ hypha (Pl. IX, Figs. 41 and 42), which ordinarily forms, upon emerging from the oospore, a number of short bristly branches (Pl. IX, Fig. 43). In water zoosporangia are generally formed, while in nutrient media, mycelial development ensues. The oospores of *P. angustatum*, n. sp., will survive conditions similar to those described for *P. adhaerens*.

IDENTITY AND RELATIONSHIP.

Of the many species of *Pythium* which have been described, the only ones with which the forms discussed in this paper need be compared to establish their identity are those in which the sporangium is filamentous throughout. This does not involve a consideration of such species as *P. aphanidermatum* (Eds.) Fitz., *P. complens*, Fischer, &c., in which the sporangium consists of a basal group of intercommunicating lobulate elements and a filamentous evacuation tube. Such forms, as has been pointed out by the writer (23), are more properly included in the genus *Rheosporangium* of Edson (11). From the monograph of Butler (6) and the papers of Pringsheim (18), Gobi (13), Schenk (20), Raciborski (19), Kanouse and Humphrey (14), Peterson (17), and Matthews (16), it is clear that there are seven species with entirely filamentous sporangia, namely, *P. monospermum*, Prings., *P. dictyosporum*, Racib., *P. afertile*, Kanouse & Humphrey, *P. papillatum*, Matt., *P. gracile*, Schenk (pro parte sensu lat. of Butler), *P. tenue*, Gobi, and *P. daphnidarum*, Pet.

From the first four species the two fungi considered in this paper are sharply distinct. In *P. monospermum*, the oospore completely fills the oogonial wall, thus differing from both of the writer's forms. *P. dictyo-*

sporum is distinguished at once by its reticulate exospore wall. *P. afertile* lacks sexual reproduction, but forms a type of spherical chlamydospore, while *P. papillatum*, by reason of its apandrous oospore which fills the papillate oogonium, is also distinct from the two forms under discussion.

Comparison with *P. gracile*, however, is difficult because, as now defined, it is sufficiently inclusive to involve several species. Of the three varieties included by Butler under *P. gracile*, all possess but one antheridium to an oogonium, while *P. adhaerens* and *P. angustatum* both exhibit a multiplicity of these organs. Further, the antheridia of the writer's two species are different in shape from the several types figured by Butler.

P. tenue differs from *P. adhaerens* and *P. angustatum* in a number of respects. Gobi has declared that no antheridial septa are present in *P. tenue*. This character alone would segregate it at once, as these were found in the writer's species. Gobi's fungus is further differentiated by the origin of its antheridia, which are mostly androgynous, as well as by the small size of its oospores ($8-9\ \mu$).

The writer's species, when compared with *P. daphnidarum*, show further individuality. As Peterson gives only a very meagre and imperfect description of his organism, an exact delimitation of it is difficult. Only one antheridium, inadequately described and figured, was found attached to an oogonium, and he was not sure of its origin. The oospores, not seen in maturity, were described as being about $22\ \mu$ in diameter. The multiplicity of antheridia of the writer's fungi distinguishes them from this doubtfully legitimate species.

It now remains to distinguish the two fungi from each other. In its vegetative structures, *P. adhaerens* is not only distinguished from *P. angustatum* by the coarseness and larger size of its mycelium, but also by the excessive production of large pyriform appressoria. Further, its stout sporangia are in marked contrast to the slender, delicate ones of *P. angustatum*. Clear cut distinctions between the two are also found in their sexual organs. The antheridial branch of *P. adhaerens*, which is never androgynous, often encircles the oogonium in a characteristic manner, its large antheridia arising as short, lateral branches of this hypha. In *P. angustatum*, while several antheridia are usually found to an oogonium, they may arise not only from a single hypha or hyphae of remote origin, but also from the oogonial hypha. No great difference in the size of the oogonium separates these two species, those of *P. angustatum* being on the whole slightly larger ($18.0-21\ \mu$, cf. $16.2-19.8\ \mu$). Further, the wall of the oospore of *P. angustatum* is always much more narrow than that of *P. adhaerens* ($1.0-1.5\ \mu$, cf. $2.0-2.5\ \mu$), a difference which was noted not only in nature, but on a variety of culture media.

For the reasons outlined above, the two forms are found to be sufficiently distinct from each other and from all other known members of the

genus to warrant their recognition as new species. The name *P. adhaerens* is therefore given to the form found on *Rhizoclonium*, referring to the adhering tendency of the appressoria; the other species is named *P. angustatum* because of its narrow oospore wall. A technical description of these species is given as follows:

P. adhaerens, n. sp.

Mycelium intra- and extramatrical; forming numerous appressoria, varying in size, although usually about $12\ \mu$ long by $7.2\ \mu$ wide; composed of hyphae $5-7.2\ \mu$ in diameter, which in the host cell present an exceedingly irregular and distorted appearance. Zoosporangium entirely filamentous, undifferentiated from the vegetative hyphae; zoospores few to many formed in a single vesicle, of the laterally biciliate type, $10.8\ \mu$ long by $6.5\ \mu$ wide; cystospore $5.4-9.0\ \mu$ in diameter, generally averaging $7.2\ \mu$, and germinating by one or two narrow germ-tubes or by repeated swarming, giving rise to another zoospore. Oogonium (on maize agar) spherical, terminal, or intercalary, $10.8-25.2\ \mu$ (averaging around $17.5\ \mu$) in diameter. Antheridium crook-necked, clavate, $15\ \mu$ long by $7\ \mu$ in the expanded distal portion, cut off by a basal septum; forming a short fertilization tube $2\ \mu$ in diameter; from one to four to an oogonium, borne on the lateral branches of a single hypha which often encircles the oogonium, or less frequently from several distinct hyphae, all of which are distinct from the oogonial hypha. Oospores (on maize-meal agar) spherical, not filling the oogonium, $7.2-21.6\ \mu$ (average $14.4\ \mu$) in diameter; containing a single oil globule; surrounded by a smooth wall $2.0-2.5\ \mu$ in thickness. Germination of the oospore not immediate, producing either a mycelium or zoospores.

Parasitic in *R. hieroglyphicum* (Ag.) Kütz., Cambridge, Mass. Capable of parasitizing, when artificially inoculated, *Spirogyra crassa*, *Ulothrix zonata*, *Tolypothrix* sp. (?), *Synedra* sp. (?), *Zea Mays*, *Pisum sativum*, *Lycopersicon lycopersicum*, *Cucumis sativus*, and *Cucurbita moschata*, as will be described in a forthcoming paper.

P. angustatum, n. sp.

Mycelium intra- and extramatrical, the latter rarely possessing small appressoria; composed of tenuous, isodiametric hyphae, $2.0-3.7\ \mu$ in diameter. Zoosporangium undifferentiated from the vegetative hyphae, delicate, and when devoid of protoplasm, tending to collapse; zoospores two to twelve in a single vesicle, of the laterally biciliate type, $8.5\ \mu$ long by $5\ \mu$ wide; cystospore averaging $6.2\ \mu$ in diameter and germinating by one or two germ tubes or by repeated swarming producing another zoospore. Oogonium (on maize-meal agar) spherical or rarely sac-like, $12.6-27.0\ \mu$ (averaging $19.8\ \mu$) in diameter. Antheridium crook-necked, $8.5\ \mu$ long by $5\ \mu$ wide, cut off by a basal septum, its somewhat blunt apex making broad

contact with the oogonium; forming a fertilization tube of varying length, approximately $2.0-2.5\ \mu$ in diameter; from one to five to an oogonium, borne terminally on lateral, narrow, distorted branches of a hypha, or from several hyphae, which are usually distinct from the oogonial hypha but may arise from it. Oospores (on maize-meal agar) one to an oogonium, sometimes two to four, not completely filling the oogonium, $10.8-25.2\ \mu$ (averaging $18.0\ \mu$) in diameter; containing a single oil globule; surrounded by a smooth, very narrow wall, $1-1.8\ \mu$ in thickness. Germination of the oospore may occur soon after formation, producing a much-branched, bristly-appearing hyphal system which may give rise ultimately to zoosporangia.

Parasitic in *S. crassa*, Kütz., Cambridge, Mass. Capable, when artificially inoculated, of parasitizing *R. hieroglyphicum*, *Oedogonium*, sp. (?), and *Zea mays*, as will be described in a later paper.

DISCUSSION.

The study of the two species of *Pythium* described in this paper involves several points of general mycological interest.

The effectiveness of *P. adhaerens* as a parasite was greatly enhanced by the formation of appressoria wherever its hyphae came into contact with the wall of the *Rhizoclonium*. Similar organs have been described in species of *Pythium* by Carpenter (7), Braun (4), Dissmann (9), and others, but their true role apparently was not appreciated, the result, no doubt, of these writers having observed them only in culture dishes, and not in nature. Adhesion organs, similar in function to those described in this paper are of common occurrence in such parasitic forms as *Polystigma*, *Colletotrichum*, *Sclerotinia*, *Botrytis*, &c. Brown (5), Blackman and Welsford (3), Dey (8), and others, have shown not only the widespread occurrence of appressoria in certain parasitic fungi, but have demonstrated unquestionably the importance of these structures in aiding in the mechanical penetration of the host cell-wall by infection hyphae.

The factor which stimulates the formation of appressoria in the two species under discussion seems to be that of contact. It does not seem likely that they are induced by lack of nourishment, as has recently been emphasized by Duke (10) in the case of *Vermicularia* and *Colletotrichum*, for it will be recalled that in *Pythium adhaerens* appressoria were also formed within the algal cell in newly attacked filaments where an abundance of food must certainly have been available. Dissmann's contention that the sickle-shaped bodies found by him in *P. proliferum* were caused by the alkalinity of the glass surface of the petri dish does not seem to apply in the case of *P. adhaerens*. For example, cultures of the latter, grown in new Petri dishes which had merely been washed, showed no excessive production of appressoria when compared with similar cultures

in dishes in which the extreme alkalinity common to new glassware had been reduced to a minimum by prolonged treatment with strong sulphuric acid. Further, as has been stated, no appressoria were formed in strongly alkalinized water, nor can the influence of alkalinity explain their occurrence on the *Rhizoclonium* walls.

Both of the forms herein described possess a very simple type of sporangium which was characterized by the older writers as 'undifferentiated from the vegetative hyphae'. After a pronounced vacuolization of the protoplasm, there are laid down at irregular intervals cross walls which delimit the mycelial segments. The latter may include one or more branches, of which the apex of one will be modified for the production of the vesicle. A mycelial segment remains morphologically a vegetative structure until one of its tips exhibits this modification, after which, it may be distinguished as a 'sporangium'. The importance of this idea of the sporangium in relation to generic concepts among pythiaceous fungi has been noted recently in Science (23) by the writer. For a discussion of the terms 'pre-' and 'pro-' sporangium, used by some authors (Edson (11), Sideris (21)) to designate this organ, the name 'sporangium' being applied only to the vesicle, the reader is referred to Fitzpatrick's comments (12), with which the writer is in entire agreement. It should be emphasized that no basal series of more or less compacted, intercommunicating lobulate elements, such as have been found in *P. aphanidermatum* (Eds.) Fitz., *P. arrhenomanes*, Drechsler, &c., was observed in *P. adhaerens* and *P. angustatum*, although a somewhat similar appearance was manifest in the former species because of the presence of intramatrical appressoria.

The vesicle formed by these fungi seems, from the writer's observations, to be derived from the expansion of the substance of the refractive apical 'dome' of the evacuation tube. The whole apical region seems to be modified for the production of the vesicular material. The vesicle in these forms is not a thin membrane resulting from the evagination of the inner wall of the sporangium, as in *Sapromyces*, *Araiospora*, *Rhipidium*, and various chytridiaceous fungi.

In the sexual stage, several points of interest are found. The precise reasons for the inconstancy in the production of sexual organs in certain cultures of *P. adhaerens* must await further investigation, although in this respect the fungus resembles certain of the homothallic Mucorales which fail to form zygospores except under very favourable circumstances.

The pronounced repeated swarming of the zoospores produced from germinating oospores of *P. adhaerens*, and the formation of small sexual organs are not regarded as being a peculiarity of this species alone, for it is quite likely that, upon further investigation, other species will be found to exhibit them. The formation in *P. angustatum* of more than one oospore in an oogonium, recalls the situation prevalent in the Saprolegniaceae. As

no such unquestionable multi-spored oogonia have ever been described in this genus, these cases possess a certain amount of interest, although their significance is problematic.

It is a pleasure to express at this time my gratitude to Professor W. H. Weston, Jr. under whose guidance this work was done, for his advice and stimulating criticism.

SUMMARY.

1. Two new species of *Pythium*, *P. adhaerens*, and *P. angustatum*, have been found parasitic in members of the Chlorophyceae collected in the vicinity of Cambridge, Mass., U.S.A.

2. The morphology and life-history of the fungi are described, the formation of appressoria and the maturation of the zoospores being dealt with in detail.

3. Oospores of *P. adhaerens* were formed, after three months, in but one of the several tubes inoculated with mycelium taken from the original colony of the fungus.

4. Zoospores produced from germinating oospores of *P. adhaerens* exhibit at least four repeated emergences. Each cystospore gives rise to 2-5 zoospores. Sex organs, unusually small, are sometimes formed on the germ hyphae of these cystospores.

5. Heterothallism, suggested by an apparent sexual segregation of the zoospores mentioned in paragraph four, and by the absence of androgynous antheridia, is not verified by the evidence derived from single spore cultures.

6. Single spore cultures derived from (a) mycelium from an oospore-producing strain, from (b) germinating oospores were sexually sterile, as were all combinations of these strains. Two months later, under as near a duplication of previous conditions as was possible, monosporic cultures from the same sources produced oospores.

7. Occasional oogonia of *P. angustatum* possess 3-5 oospores.

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EXPLANATION OF PLATE IX.

Illustrating Dr. F. K. Sparrow's paper on Two New Species of *Pythium* Parasitic in Green Algae.

The figures were drawn from living material with the aid of the camera lucida. The approximate magnification of the drawing is given in each case, but applies to the original figures which have been slightly reduced in reproduction.

Pythium adhaerens, n. sp.

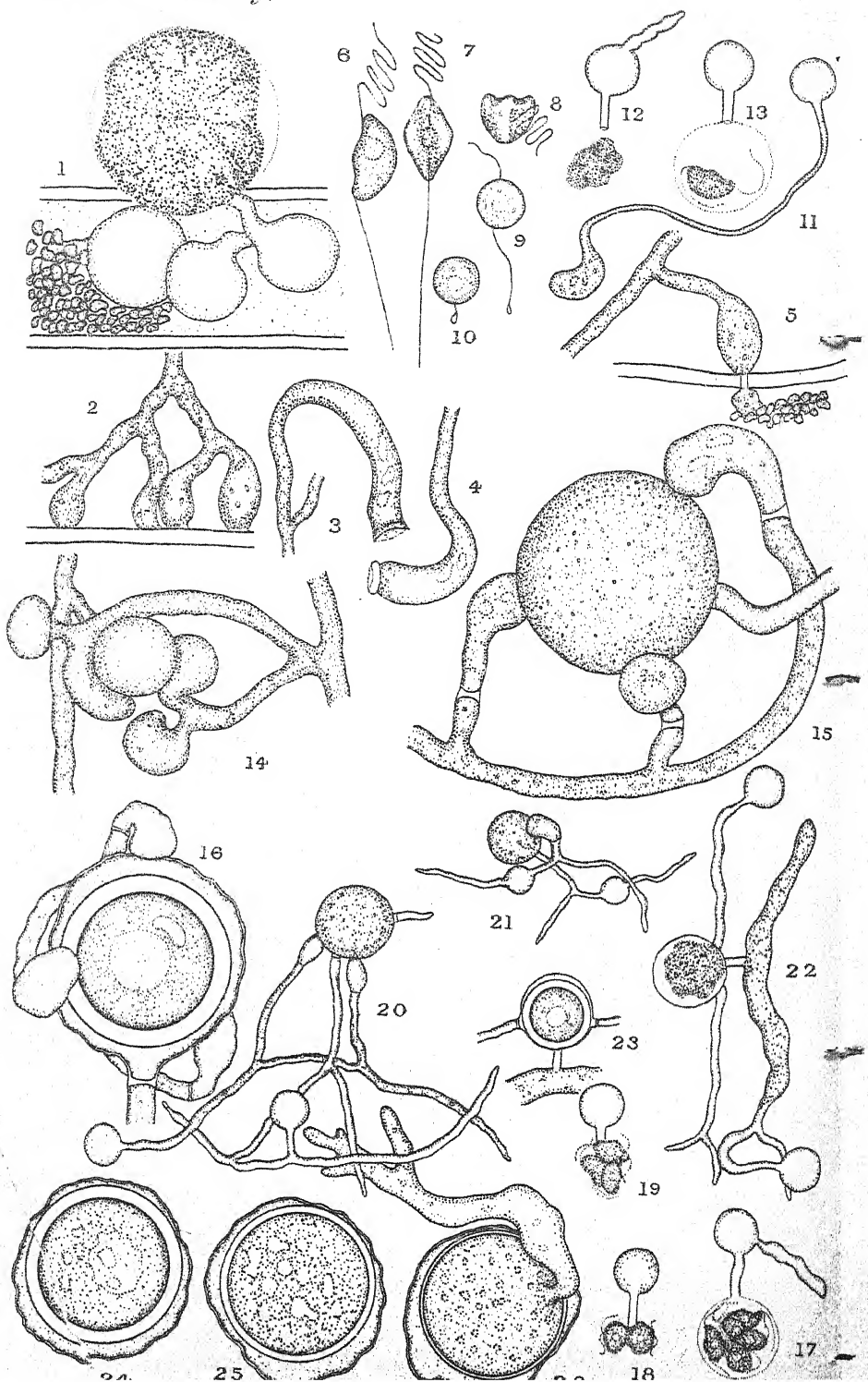
Fig. 1. Zoosporangium, in *Rhizoclonium*, which has ejected its content into the vesicle formed outside of the algal cell. $\times 1,200$.

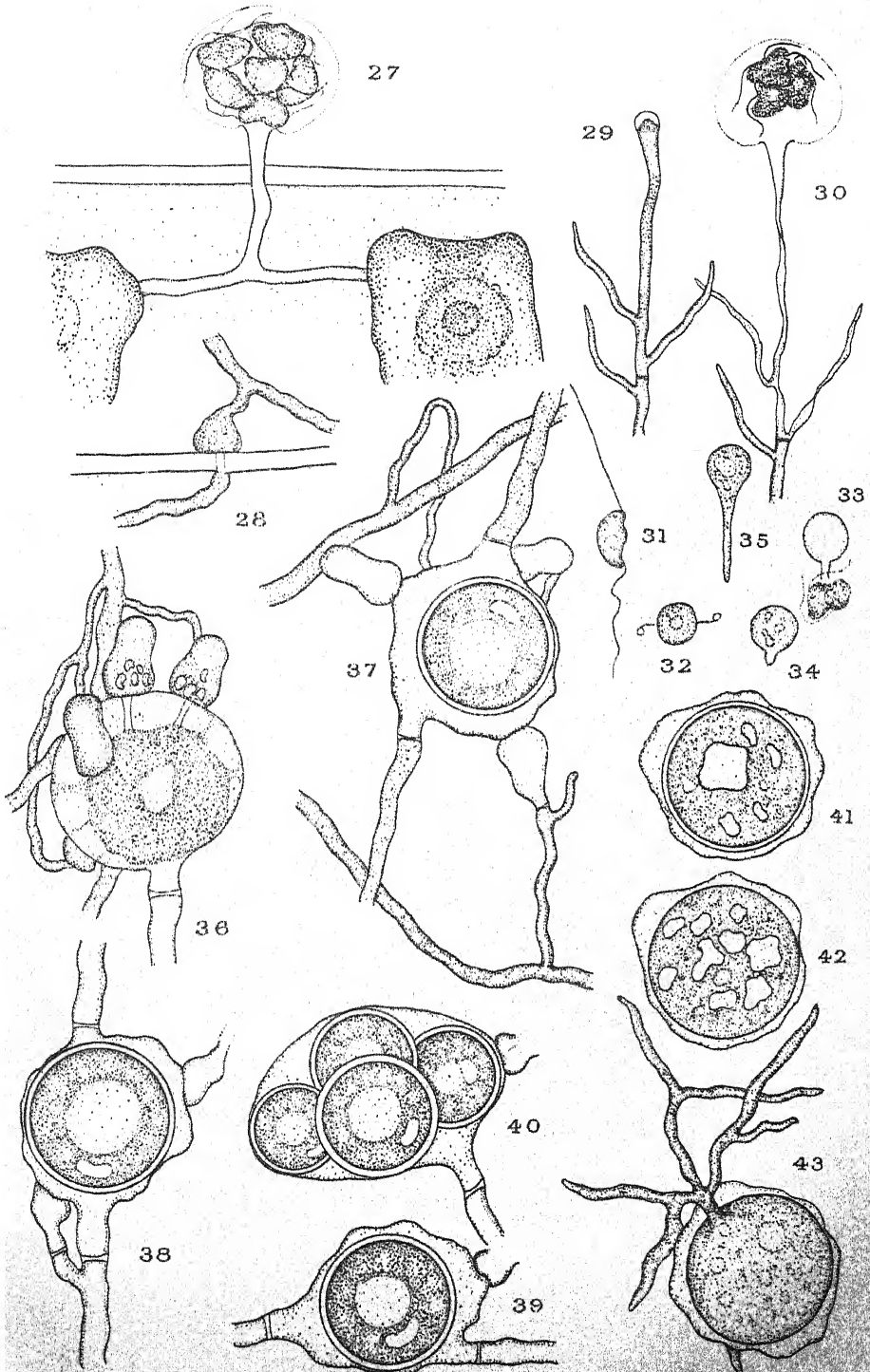
Fig. 2. Cluster of extramatrical appressoria attached to the algal wall. $\times 1,200$.

- Figs. 3, 4. Sickle-shaped appressoria formed upon contact with the surface of culture dishes. The apical disk of mucilaginous material is clearly shown. $\times 1,400$.
- Fig. 5. Appressorium, showing penetration tube and depressed wall of the alga. $\times 1,200$.
- Figs. 6, 7, 8. Side, top, and front views, respectively, of the zoospore. $\times 2,220$.
- Figs. 9, 10. Stages in the withdrawal of the cilia. $\times 2,200$.
- Fig. 11. Germinated cystospore, showing a small appressorium formed at the tip of the germ hypha. $\times 2,200$.
- Figs. 12, 13. Stages in the repeated emergence of the zoospore. In the first instance, no vesicle could be observed. $\times 2,200$.
- Fig. 14. Early stage in the development of the sexual organs. $\times 2,200$.
- Fig. 15. A later stage. $\times 2,200$.
- Fig. 16. A mature oospore. $\times 2,200$.
- Figs. 17, 18, 19. Repeated emergence of zoospores from germinated oospores. $\times 1,500$.
- Fig. 20. Sexual organs formed by the germ hyphae of zoospores. $\times 1,500$.
- Fig. 21. A similar case, showing only one antheridium. $\times 1,000$.
- Fig. 22. In this instance no antheridium was formed, a hyaline tube from one hypha penetrating the oogonium and discharging a portion of the content. $\times 1,500$.
- Fig. 23. Oospore formed after fertilization by the oogonium shown in the preceding figure. $\times 1,500$.
- Figs. 24, 25, 26. Stages in the germination of the oospore. $\times 2,200$.

P. angustatum, n. sp.

- Fig. 27. Zoosporangium protruding from a portion of a cell of *Spirogyra*. $\times 1,600$.
- Fig. 28. An appressorium adnate to the wall of the alga, showing the penetration tube and intramatrix hypha. $\times 1,600$.
- Fig. 29. Zoosporangium just prior to discharge. $\times 1,500$.
- Fig. 30. Zoosporangium after discharge; zoospores nearly matured. $\times 1,500$.
- Fig. 31. Side view of zoospore. $\times 1,500$.
- Fig. 32. Zoospore retracting its cilia. $\times 1,500$.
- Fig. 33. Repeated swarming of the zoospore. $\times 1,500$.
- Figs. 34, 35. Stages in the germination of the cystospore. $\times 1,500$.
- Fig. 36. Sexual organs during the process of fertilization. $\times 2,200$.
- Fig. 37. Mature oospore, showing antheridia of declinuous origin. $\times 2,200$.
- Fig. 38. Oospore, showing antheridia arising from the oogonial hypha as well as from one of distant origin. $\times 2,200$.
- Fig. 39. Oospore formed from a sac-like oogonium. $\times 2,200$.
- Fig. 40. A multi-spored oogonium. $\times 2,200$.
- Figs. 41-3. Stages in the germination of the oospore. $\times 2,200$.





Mechanism of Curvature in the Tendrils of Cucurbitaceae.

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With twenty-two Figures in the Text.

BOTANISTS have been attracted from the middle of the nineteenth century to the phenomenon of curvature of the tendrils of the climbing plants, when they come in contact with a solid support. Of these contact curvatures exhibited by tendrils, those tendrils which show curvatures within a fraction of a minute are particularly of great interest. The study of this type of contact curvatures can be said to have begun from 1827 when Hugo von Mohl (11) first noticed the curvature of tendrils on contact irritability. Later, Dutrochet (3) studied the general behaviour of climbing plants, but a very extensive study of the physiology of twining was made by Darwin (1). Pfeffer (15), by his detailed researches on the thigmotropic irritability of tendrils, showed that the blows inflicted on the tendrils by liquids failed to act as stimuli, while the blows inflicted by solid objects in rapid succession acted as stimulants. He concluded that a statical pressure did not act as a stimulant, but the sensitive region of the tendrils must be rubbed with an uneven surface in order to cause the curvature of tendrils.

There has been great difference of opinion on the causes of the curvature of tendrils on the application of a stimulus. On account of the rapidity with which the tendrils of Passifloraceae curled on a contact stimulus, Darwin (1) thought that curvature of tendrils was in some cases at least due to the contraction of the concave side, and it was not due to accelerated growth of the convex side as was first put forward by Sachs (16) for all twining and tendril-bearing plants. Fitting (4) confirmed the view held by Sachs (16) from the study of the mechanism of curvature of tendrils, and concluded that the curvature of tendrils could be attributed to growth, and not to the fall in turgidity in the cells on the concave side, as was also

held by MacDougal (9,10). The observations of de Vries (2) did not support the view held by Sachs (16) and Fitting (4), as he failed to notice the accelerated rate of growth on the convex side. De Vries (2) maintained that contact stimulus produced a rise of turgor on the convex side which underwent an elastic stretching made permanent by subsequent growth.

The study of the physiology of curvature has been made from two points of view. Firstly, it is attempted to explain the nature of the stimulus necessary for contact irritability, and how it is perceived, and secondly, the causes leading to the curvature are investigated.

Haberlandt (5) has increased our knowledge on two important points. He has shown from anatomical studies that the perception of the contact stimulus takes place through certain specialized epidermal cells, known as tactile pits which function as organs of perception. These pits are formed in the outer walls of the epidermal cells, and the protoplasmic layer penetrates into these pits. The protoplasm is irritated when they brush against a solid object.

These observations of Haberlandt (5) have recently been extended by Kanga and Dastur (7), and they have conclusively shown the presence of such pits in some of the plants growing here. They have shown the different stages of the modification of the outer epidermal walls as sense organs, from a rough outer surface as in the species of *Vitis* to specialized pits in the outer epidermal walls as in *Luffa acutangula*, Roxb.

Haberlandt (5) favours the view of Sachs (16) and Fitting (4) that the curvature of tendrils depends upon the accelerated growth on the convex side. But he has shown that in the tendrils of *Urvillea ferrugenea* the curvature is due to the contraction of certain living cells forming a motor tissue on the concave side.

In spite of the investigations of the above-mentioned writers the exact mechanism of the thigmotropic curvatures is not clear. Attempts up to now have been made to explain the curvatures by investigating the anatomical features, or by measuring the growth on the two sides of the tendrils before and after the curvature, but they do not throw any light on the principle on which the mechanism of curvature depends. The experiments carried out so far to demonstrate that the curvature is due to unequal growth on the convex and concave sides of the tendrils are open to objections. The increase in the distance between the ink-marks before and after curvature, does not tell us whether the convex side has actually grown in length, or the cells on the convex side have merely undergone elongation. Even fully grown tendrils, when the growth in length has ceased, can be noticed curling round a support and forming spirals.

The measurements of the lengths of the cells on the convex side, before and after the curvature, as is done by some writers to prove that the curvatures are caused by the elongation of the convex side, and the con-

traction of the concave side, require very careful considerations of certain points in the method employed. The average dimensions of the cells vary from the base towards the apex, and also according to the age of the tendrils. In order to find out the differences in the lengths of the cells in an uncurled and curled tendril, it is not possible to make the observation on the same tendril, but on two different tendrils, so it is necessary to select the two tendrils which are of the same length and of the same age, and the portions selected for the measurements of the cells should be at equal distance from the apices of the two tendrils. If that is not done the results obtained are not comparable.

It would thus be of interest to study the mechanism of thigmotropic curvatures in tendrils. It is possible that the curvatures are not due to the same cause in the tendrils of plants of different families. The mechanisms of curvature may be different in different plants. It is well known that the tendrils of Cucurbitaceous plants are sensitive. They show visible response to the contact stimulus within a few seconds, and the anatomy of these tendrils has been studied by various workers, especially by Penhallow (14), Worgetzky (17), Muller (12), and Lisk (8).

Recently the physiological anatomy of Cucurbitaceae has been studied by Holroyd (6) and also by Zimmerman (18). The latter writer (19) has published a book dealing with the anatomical and physiological investigations of Cucurbitaceae, and the morphological nature of the tendrils has been discussed by Neitsch (13), but no observations were made regarding the physiology of curvature of the tendrils. Kanga and Dastur (7) in their study of the physiological anatomy of climbing plants have included two species of *Luffa* amongst the Cucurbitaceous plants, and have described their anatomical structure.

There are a large number of Cucurbitaceous plants growing wild in the Bombay Presidency, and the sensitiveness of their tendrils to the stimulus of contact is very well known. So it was decided to investigate the mechanism of curvature in these tendrils, and to determine whether it is the same in all cases, or if it differs amongst the species of the same family. Different views have been expressed regarding the mode of curvature in the different species of Cucurbitaceae studied by different writers mentioned above, and therefore a thorough investigation of a number of species would be of special advantage in tracing the causes which give rise to such rapid curvatures.

The different species of Cucurbitaceae were grown from seeds in the garden of the Institute. The Cucurbitaceous plants growing here are mostly annuals, and therefore they are available for observation during the monsoon for about five months in a year, from July to November. All the observations recorded below were made in these months of 1926, 1927, and 1928.

INVESTIGATION.

It was first thought necessary to measure the growth in length of the tendrils in order to determine if any correlation existed between the age of the tendrils and their respective lengths, so that the tendrils of the same age, as well as fully grown tendrils, could be taken for further investigations. The growth in length of different tendrils of a number of species in different stages of growth was measured every day. In the following table some of the results are given.

TABLE I.

Tendrils number.	Initial length of the tendril.	Length after 24 hours.	Length after 48 hours.	Length after 72 hours.
<i>Momordica Charantia</i> , L. (main shoot).				
	cm.	cm.	cm.	cm.
1	3.4	8.1	17.5	19.5
2	7.3	15.3	18	19.5
3	15	18	19.2	19.2
4	18.5	19.3	19.5	19.5
<i>Lagenaria vulgaris</i> , Seringe. (main shoot).				
1	9.5	26.7	32.5	37.3
2	23	32.5	37.2	37.3
<i>Cucumis sativus</i> , L. (side branch).				
1	8.5	10	11.3	17.9
2	13.1	16.9	17.7	18
<i>Cucurbita Pepo</i> , L. (side branch).				
1	17.2	18.6	19.5	19.8
2	17	18.8	19.1	19.5

} 1st branch of the
tendril.

The tendrils of one of the main shoots of *Cucurbita Pepo*, L. attain a length of 29.7 cm. (In rare cases they are as long as 36 cm.) Similarly the tendrils of one of the main shoots of *Citrullus vulgaris*, Schrad., attain a length of 10 cm., while those of the side branches are 6.7 cm. long when they are fully grown. In the case of *Luffa acutangula*, Roxb., the five branches of the tendrils acquire their respective lengths when they are fully grown. The longest reaches a length of about 26 cm., the two longer branches reach a length of about 14 and 11 cm., and the other two about 9 cm.

Tendrils of *Momordica Charantia*, L., is fully grown when it reaches a length of 19.2 to 19.5 cm. It appears to be a very fast growing tendril, reaching its maximum within four days. The tendrils of *Lagenaria vulgaris*, Seringe, is fully grown at 37.3 cm., and is equally rapid in growth as those of *M. Charantia*, Linn. The tendril of *C. Pepo*, L., is not simple,

but it has three branches. The growth in length of each of the three branches is measured. It is seen that each branch attains its own specific length. The three branches have different lengths when they are fully grown. The first branch attains a length of 19.8 cm., the second 10 cm., and the third about 6 to 6.5 cm.

TABLE II.

	Initial length of the tendril.	Increase in length in 24 hours.	Increase in length in 48 hours.	Increase in length in 72 hours.
<i>Momordica Charantia</i> , Linn.				
	cm.	cm.	cm.	cm.
	3.4	4.7	9.4	
	7.3	8	4.2	
	15	3	1.2	
	18.5	0.8	0.2	
<i>Lagenaria vulgaris</i> , Seringe.				
	9.5	17.2	5.5	5.1
	23	9.5	4.7	0.1
<i>Cucurbita Pepo</i> , L.				
1st Branch	17.2	1.4	0.9	0.3
2nd "	7	1.3	1.7	0.5
3rd "	3.5	1	1.6	0.7
1st "	17	1.8	0.3	0.4
2nd "	8	0.5	0.7	0.3
3rd "	3.8	0.4	0.3	0.3

It is evident from the above figures that the growth in length is at its maximum when the tendril grows 8 to 9.4 cm. in 24 hours, in *M. Charantia*, Linn., and 17.5 cm. in *L. vulgaris*, Seringe. If we start with a young tendril, the period of maximum growth is on the second day of the observations, while in a slightly older tendril it is on the first day. So it passes through the maximum phase of elongation when it reaches a certain length.

The tendril of *C. Pepo*, L. has a stalk from which three branches arise. Out of the three, one is longest, the second is of moderate length, and the third is the smallest of the three. All the three grow with different rates and attain different lengths at maturity.

In the case of *M. Charantia*, L., when the total increase in length in twenty-four hours was measured, marks were made at the apical region at a distance of 2 cm. each. The first mark being made at 1 cm. from the apex, and the increase in length between the marks was measured. In the following table the increase in length of each 2 cm. is given. In the table below the numbering of marks is made from the apex. In the first tendril only two marks could be made as the tendril is only 3.4 cm. long.

TABLE III.

Momordica Charantia, L.

Initial length of the tendril.	1st mark.	2nd mark.	3rd mark.	Increase in length in 24 hours in each division of 2 cm.		
				4th mark.	5th mark.	6th mark.
cm.	cm.	cm.	cm.	cm.	cm.	cm.
3.4	0.6	1.1				
7.3	0.8	1	1.2	1.2		
15	0.2	1.6	3	0.2	0.5	0.4
18.5	0.1	0.1	0.1	0.1	0.1	

So in *M. Charantia*, L., the growing region is confined at the apex for about 10 cm. The basal region does not show any appreciable increase in length. The maximum growth is taking place about 3 cm. from the apex. As the tendril becomes mature the growth at the apex falls, as is seen in the fourth tendril. The same is true of the tendrils of *C. sativus*, L. The growing region extends up to 11 cm. from the apex.

TABLE IV.

Cucumis sativus, L.

Increase in length of each division.

	Initial length of the tendril.	1st mark.	2nd mark.	3rd mark.	4th mark.	5th mark.	6th mark.	7th mark.
		1 cm.	2 cm.	2 cm.	2 cm.	2 cm.	2 cm.	2 cm.
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
In 24 hours	13.1	0.5	1	0.7	0.5	0.3	0.5	0.1
" 48 "		0.2	0.5	0.3	0.1			
" 24 "	14	0.2	0.2	0.1	0.2	0.1		
" 48 "		0.1	0.1	0.1				
" 24 "	12	0.5	0.9	0.5	0.5	0.6	0.6	

Similar measurements were made for *Coccinia indica*, Wight and Arn., and *C. Melo*, Linn., which have identical results. But in the case of *L. vulgaris*, Seringe, the growth is not restricted at the apex, but the whole tendril grows in length. The basal region grows more rapidly than the apical region. This point was first discovered by marking the tendril and making the measurements of the marks after twenty-four hours.

The tendril has a stalk and two branches. The stalk also grows in length along with the branch.

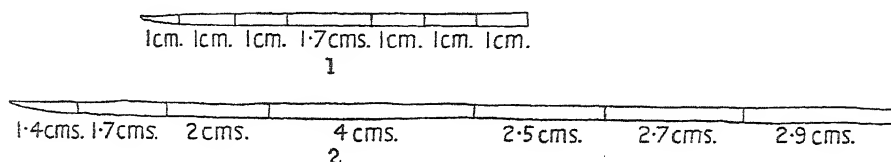
TABLE V.

Lagenaria vulgaris, Seringe.

Initial length of the stalk.	Increase in length in 24 hours.	Total length.
cm.	cm.	cm.
1.5	5	6.5
5	2.5	7.5
2.7	4.5	7.2

The maximum length attained by the stalk is 7.5 cm.

The two branches of the tendrils were marked at 1 cm. apart, and the following increases in length between the marks were noticed. Some marks were made at the apex, and some near the base as shown in the Figs. 1 and 2.



FIGS. 1 and 2. 1. A young tendril of *Lagenaria vulgaris*, Seringe, marked in centimetres. Natural size. 2. The same tendril (Fig. 1) after twenty-four hours, showing growth in length between the marks. It shows very marked growth at the basal region. Natural size.

The following table shows the increase in the distance between the marks of 1 cm. each at the base and the apex.

TABLE VI.

Lagenaria vulgaris, Seringe.

	Initial length of the tendril. cm.	Increase in 24 hours in each division of 1 cm. from the apex.				
		1st. cm.	2nd. cm.	3rd. cm.	4th. cm.	5th. cm.
1st branch of the tendril {	14	0.2	0.4	0.6	0.6	0.8
2nd branch of the tendril {	7.7	0.4	0.7	1		
	5.5	0.5	0.9			
	3.6	0.4	Increase in 24 hours in each division of 1 cm. from the base.			
1st branch of the tendril {	14	1.7	1.3	1.2	0.9	1.4
2nd branch of the tendril {	7.7	1.9	1.7	1.5		
	5.5	1.8	1.36			
	3.6	0.9				

The middle region of the two first branches of the tendrils 14 cm. and 7.7 cm. in length, grow from 4 cm. and 1.7 cm. to 7.8 cm. and 4 cm. respectively. In the two second branches of the tendrils the middle regions grow from 1.5 cm. and 1.6 cm. to 2.9 cm. and 2.8 cm.

It is evident from the figures that the growth at the base is more vigorous than the growth at the apex.

The next point that was considered was the period at which the tendril becomes sensitive to contact. It was first tried to find out at what stage in growth the tendril shows sensitiveness to contact even in a slight degree. In the case of *M. Charantia*, L., the sensitiveness to contact is shown by a tendril when it is 7 cm. long, i.e. when it has attained one-third of the maximum length. In young tendrils the curvature disappears after some time. As the tendril grows in length the sensitivity increases, and when it is

full grown it is the most sensitive. The following table shows the reaction time of different tendrils of some of the plants examined to show visible curvature after stimulation by gentle rubbing on the concave side.

TABLE VII.

Initial length of the tendril.	Reaction time.	Initial length of the tendril.	Reaction time.
<i>Momordica Charantia</i> , L.		<i>Cucumis sativus</i> , L.	
cm.	seconds.	cm.	seconds.
9	90	10.5	120
16	30	25.5	60
17.7	22	33	50
19	20	34.5	32 to 40
<i>Cucurbita Pepo</i> , L.		<i>Benincasa cerifera</i> , Savi.	
4.5	120	9.3	60
8.5	90	13.2	60
14.2	60	18.5	50
18.2	40	24	30
19.5	60		
24.5	60	29.0	30
21.5	60	30	35
		31	31

In the case of *C. sativus*, L., the tendril becomes sensitive to contact when it reaches a length of about 9 cm. and the reaction time depends upon the age of the tendril. In the case of *C. Pepo*, Dc., the maximum sensitivity is attained when the tendril is about 19 cm. long. Similar observations were made on the tendrils of *C. indica*, Wight & Arn., *C. Melo*, Linn., *Luffa acutangula*, Roxb., and other species.

TABLE VIII.

Initial length of the tendril.	Time taken to form one complete coil.	Initial length of the tendril.	Time taken to form one complete coil.
<i>Momordica Charantia</i> , L.		<i>Cucumis sativus</i> , L.	
11 cm.	10 minutes	10.5 cm.	No coil formed
12.4 "	9 "	25.5 "	5 minutes
14.2 "	6 "	34.5 "	1 "
15.2 "	5 "	33 "	1 "
15.5 "	5 "		
<i>Benincasa cerifera</i> , Savi.			
9.3 "	No coil formed		
13.2 "			
18.5 "			
24 "	2 minutes		
29.5 "	2 "		
30 "	2 "		
31 "	1½ "		

The time taken by the tendril to form one complete coil was then measured after the stimulus of contact was applied as in the above experiments. It was noticed that the time taken to give rise to one perfect coil at the apical region varied according to the age of the tendril.

Young tendrils, though sensitive to contact, do not form complete coils on stimulation, while older tendrils form complete coils within a few minutes after the stimulus is applied. The formation of coils is thus related to the length, and consequently to the age of the tendril.

The time taken by a tendril to curl round a support was then measured. The support in all cases was a piece of glass rod of 0.5 cm. in diameter. The length of the tendril was first measured, and the time taken to form one complete coil round the support was recorded. The tendril was then left with the support for twenty-four hours. The next day the number of coils round the support and the number of spirals formed in the free portion of the tendril were determined. The observation was repeated after forty-eight hours.

On examining the data of Table IX it is clear that the number of coils formed by a tendril round a support depends upon its age. In the case of *L. vulgaris*, Seringe, if a tendril 13 cm. in length happens to come in contact with a support the number of coils round the support would be four, but if a tendril 25 cm. in length reaches a support the number of coils round the support is two. Similarly, the number of spirals formed in the free portion is greater with the greater length of the tendril. The same is the case in *M. Charantia*, L., *C. Pepo*, L. and *C. indica*, Wight & Arn. In all the above cases the tendrils were made to touch the support at the tips. It is evident that the number of cells formed round the support is due to the growth of the tendril, a young tendril forming more coils round the support than a mature tendril.

In order to test this point that the number of coils formed round the support by a tendril depends upon its age, fully-grown tendrils were selected. The age of the tendril was determined by its length. It was made to hold a support near the tip and ink marks made on it at 2 cm. apart near the tip. It formed a hook within 15 minutes, and was allowed to remain attached to the support. The next day the tendril was examined, and it was found that it had taken only one complete coil round the support, and no more coils were formed later on. The distance between the marks had not appreciably increased. It indicated that the growth in the tendril had ceased, and therefore no more coils were formed round the support. Another full-grown tendril of *L. vulgaris*, Seringe, was similarly brought into contact with a support, but this time not at its tip, but at about 5 cm. below the tip. The tendril is sensitive at that distance from the apex, and it began to curl within 60 seconds, and one coil was completed in 15 minutes. It did not take a second spiral, but next morning the apical region was

coiled on itself, or in another experiment coiled loosely, round the support, which is tightened by growth in thickness and by straightening. This is a point of interest, as the number of coils round a support will also depend upon the point at which the tendril makes contact, but here the coiling is not due to growth in length, but only due to curvature of the tendril like the formation of spirals.

TABLE IX.

Initial length of the tendril.	No. of coils round the support after 24 hours.	No. of spirals and reversion points in the free portion after 24 hours.	No. of spirals and reversion points after 48 hours.
<i>Lagenaria vulgaris</i> , Seringe.			
cm.			
13.2	4	2 spirals with one reversion point in the middle.	12 spirals with 1 reversion point.
16.5	4	6 spirals with 2 re- version points.	12 spirals and 2 reversion points.
19.5	2	12 spirals with 4 reversion points.	No change.
25	2	16 spirals with 1 reversion point.	20 spirals with 1 reversion point.
<i>Momordica Charantia</i> , L.			
7	4	5 spirals with 1 re- version point.	
10.5	2	5 spirals with 1 re- version point.	
13.5	2	10 spirals with 2 reversion points.	
<i>Cucurbita Pepo</i> , L.			
15	3	15 spirals with 2 reversion points.	17 spirals with 2 reversion points.
17.5	3	12 spirals with 1 reversion point.	13 spirals with 1 reversion point.
21.2	3	12 spirals with 1 reversion point.	No change.
28.8	2	14 spirals with 1 reversion point.	No change.
31.2	1	12 spirals with 2 reversion points.	17 spirals with 4 reversion points.
<i>Coccinia indica</i> , Wight & Arn.			
7.5	4	8 spirals with 2 re- version points.	15 spirals with 2 reversion points.
11.5	3	9 spirals with 2 re- version points.	21 spirals with 4 reversion points.

It is also observed that if a young tendril happens to catch a support near the apex it takes a few coils around it, but on measurement of the length after coiling has ceased, it is found that the tendril had not attained its usual length. The growth in length of the tendril is arrested on account of the curvature. It is very likely that the cells which are responsible for growth lose their meristematic activity on account of the strains of compression and extension after the curvature.

In some cases the apical region, after two or three coils round the support, continues to grow in length in a straight line, and if it catches another support it curls round it. In this way the same tendril can catch two supports.

The above investigation has brought out the following facts regarding the physiology of the tendrils of Cucurbitaceae :

(1) The tendril, like other organs of a plant, undergoes a grand period of elongation. In some tendrils the growth in length is confined to the apex, while in others the basal region grows in length more vigorously than the apical region.

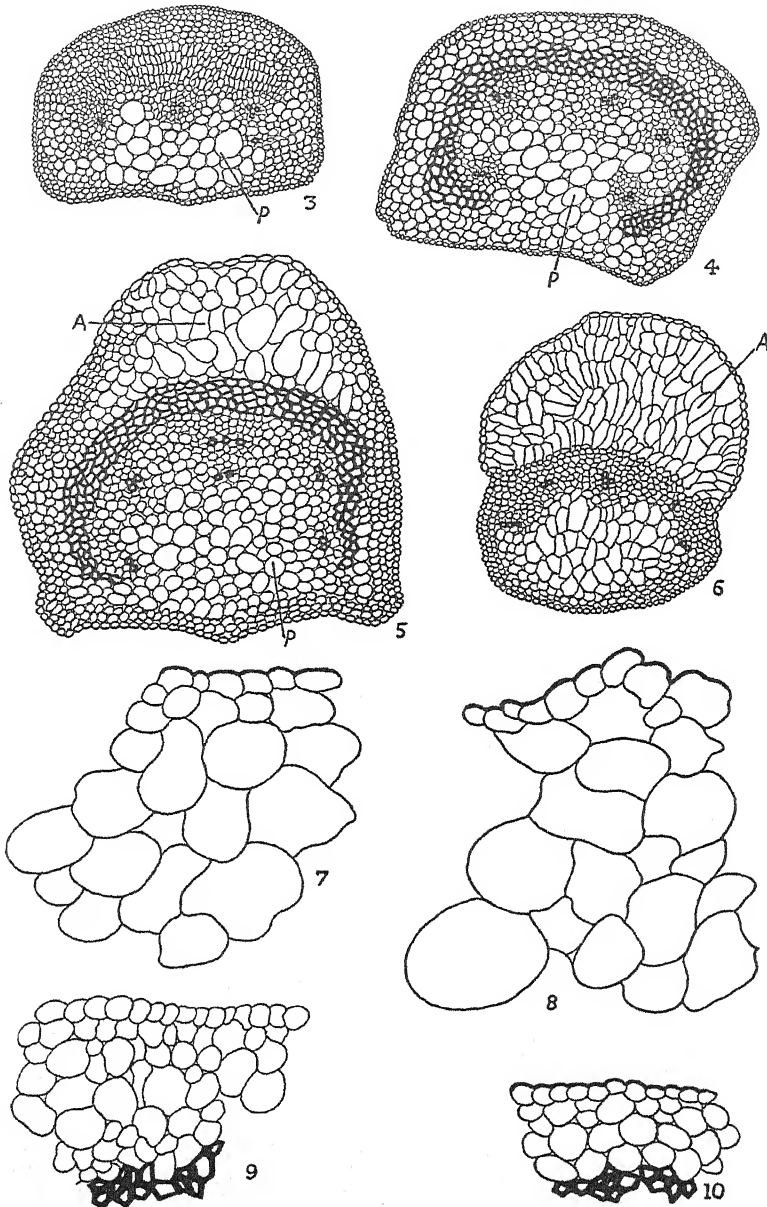
(2) The tendrils generally become sensitive before they attain one-third of their specific lengths.

(3) The reaction time varies from 20 seconds to 2 minutes after the stimulus is applied. It varies according to the age of the tendrils. The reaction time of some tendrils becomes less as they grow more in length, while in other cases it becomes greater.

(4) The time taken to form one complete coil also varies according to the age of the tendril.

(5) The number of coils round the support depends upon the age of the tendril. If a young tendril happens to catch a support the number of coils is greater than in the case of a full-grown tendril.

It is evident that the number of coils round a support depends upon the age of the tendril, and it is also clear that the greater number of coils round the support means the greater growth in length. A young tendril, like a twining stem, both curls and grows in length. That the curling is not due to unequal growth on the two sides has been brought out by the above experiments. A fully-grown tendril never takes more than one coil round the support. Secondly, if it is made to catch a support below the apex, one single coil is formed round a support, and the apical region forms spirals on itself or round the support, and there is no appreciable increase in length. The curvature necessary for curling round the support, and for the formation of spirals, which are also caused by curvature of the tendrils, is due to some other cause than unequal growth, because such curvatures are exhibited by the tendrils which have ceased growing in length. *A tendril may curl as it grows, but it does not curl as a result of growth.* Furthermore, it is difficult to conceive of such rapid growth



FIGS. 3-10. In Figs 3-6 the concave side is towards the top of the plate. *p.* = parenchymatous cells which elongate on the convex side; *a.* = abnormal growth of cells on the concave side after a support is caught. 3. Transverse section of a young tendril of *Citrullus vulgaris*, Schrad. $\times 50$. 4. Transverse section of an old curled tendril of *Momordica Charantia*, L. $\times 30$. 5. Transverse section of a curled tendril of *Momordica Charantia*, Linn., showing abnormal growth on the concave side after curling round a support. $\times 50$. 6. Transverse section of a curled tendril of *Cucumis Melo*, L. 7. Transverse section of a curled tendril of *Cucumis Melo*, L., showing

on the convex side in the course of a fraction of a minute. That curvature is not caused by unequal growth is supported by the investigations described below:

The tendrils of Cucurbitaceae, it has been observed, are as a rule coiled like a watch-spring in the young stage of growth, and the side that becomes the convex one in the stimulated curled condition is concave at that stage and vice versa. Then the growth becomes more vigorous on the inner side than on the outer, so the tendril begins to open out and becomes straight. The tendril then grows uniformly on both sides, except for a slight curvature at the tip. On examination of these tendrils it is found that the tendrils are bilateral, and the side that becomes the convex side has a groove in the centre, while the side that becomes the concave side is bulged out, as is evident from its cross section (Fig. 5). The peculiarity of these tendrils is that, when they coil round the support, or when the spirals are formed, the grooved side is always the convex side, and the bulged side is always the concave side. This is not the case for tendrils in general, as any point on the surface may be towards the concave side in course of curving. But in this case the convex and concave sides are, as it were, pre-determined, and the curvature takes place in such a manner that the grooved side never becomes the concave side or the bulged side the convex. This leads to the conclusion that the internal structure of the tendril may have something to do with the mechanism of curvature, and the character of the tissues on the two sides of the tendril is such that the curvature can take place in one plane only. The causes that bring about the curvature of a tendril round the support must also be responsible for the spirals formed in their free portions.

The tendrils of all the Cucurbitaceous plants so far examined are bilateral in symmetry. The cross-section of a very young uncurled tendril shows very little differentiation of tissues, except for a faint indication of xylem elements. As the tendril grows older the differences in the cells on the convex and the concave sides become visible. The epidermal cells contain protoplasts, and in the epidermis of the tendrils of *Momordica Charantia*, L., *Cucurbita Pepo*, L., *Cucumis Melo*, L., *Citrullus vulgaris*, Schrad, &c., the tactile-pits are seen. Two rows of cells below the epidermis both on the concave and convex sides after the tendrils are fully grown consist of living elements. The remaining cortical cells on the concave side outside the sclerenchyma band are small and slightly thick-walled (Fig. 3). The sclerenchyma band is not developed in an uncurled tendril, but the

the parenchymatous cells on the convex side. $\times 450$. 8. Transverse section of a curled tendril of *Mukia scrabrella*, Arn., showing the parenchymatous tissue on the convex side. $\times 450$. 9. Transverse section of a curled tendril of *Cucumis Melo*, Linn., showing cells on the concave side. $\times 450$. 10. Transverse section of a curled tendril of *Mukia scrabrella*, Arn., showing cells on the concave side.

cells which are to become sclerenchymatous could be easily differentiated under the microscope (Fig. 3). Inside the sclerenchymatous band lie the vascular elements. The primary bundles are generally five in number, but the number varies from five to seven. The two vascular bundles lie on the two projections on the convex side, while the rest lie lining the sclerenchymatous band (Fig. 4) on the concave side. The rest of the tissue in the central part of the tendril and on the convex side is made up of thin-walled large parenchymatous cells (Fig. 5).

The tendrils of the different species of the following genera were examined anatomically in this investigation: 1. *Momordica*. 2. *Luffa*. 3. *Cucumis*. 4. *Melothria*. 5. *Cucurbita*. 6. *Benincasa*. 7. *Mukia*. 8. *Coccinia*. 9. *Trichosanthes*. 10. *Lagenaria*. 11. *Citrullus*.

All the species have the same type of structure as described above. In old and curled tendrils the sclerenchymatous band is fully developed (Figs. 4 and 5).

After a tendril has curled round a support it is noticed that after some time the cortical cells on the concave sides begin to enlarge in the radial direction, and some of them divide actually. In this way a mass of tissue of radially elongated cells is formed on the concave side, as is seen in the Figs. 5 and 6 a.

The stimulus of contact is perhaps the cause of this secondary development; it gives additional mechanical strength to the tendril.

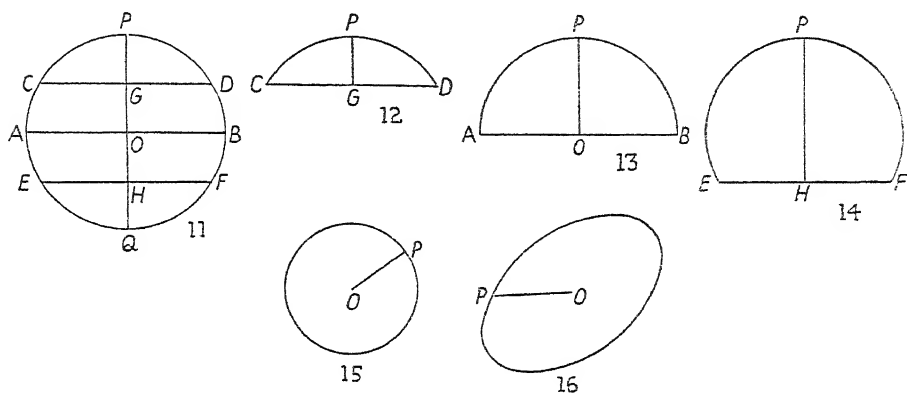
On examination of the cross-section it became evident that the arrangement and the nature of the tissue on the two sides of the tendrils are based on mechanical principles. When a tendril curves the concave side is in a state of compressibility, and the convex side is in a state of extensibility. So the cells on the concave side bear the strain due to compression, and the tissue on the convex side has to bear the strain of extension. For this reason the dense material in the form of small cells (Figs. 9 and 10) is located on the concave side, together with the mechanical flange in the form of sclerenchymatous cells, while the extensible tissue in the form of large wide cells is situated towards the convex side (Figs. 7 and 8). In order to obtain the quantitative data regarding the density of material on the concave and convex sides the following method was applied. The density of material depends mainly on the cell-wall material, and so it was thought necessary to find out the quantity of cell-wall material in a unit area on the concave and convex sides.

Camera lucida drawings were made of the cells on the concave and convex sides for a unit area. The method by which the sum of the perimeters of the cells in a unit area can be determined depends upon a calculation as certain cells are circular, certain elliptical, and some are portions of circles.

Consider a circle (Fig. 11) of any radius a . AB and PQ are two

diameters cutting each other at right angles at O. Bisect PO in G and OQ in H. Draw CD parallel to AB and EF parallel to AB. Consider now the following forms separately.

If any curve (i. e. part of a cell) corresponds more to Fig. 12 than to Figs. 13 and 14 then the length of the arc CPD in Fig. 12 is $4\frac{3}{4}$ times PG.



FIGS. 11-16. For explanation see the text.

If any case corresponds to Fig. 13 then the length of the curve APB is 3 times OP.

If the form of the curve corresponds to Fig. 14 then the length of the arc is $2\frac{3}{4}$ times PH.

In the case of an oval we take an apparently mean diameter OP and define the length of the oval as $6 \times OP$ (Fig. 16).

In the case of a circle the length of the circle is $6 \times OP$ (Fig. 15).

How this method is actually applied to determine the sum of the outlines of the cells on the concave and convex cells is given below.

Drawings are made under the camera lucida of the cells of the concave and convex sides, and the magnification of the drawing measured. The cells situated in 0.25 of a square inch of the drawing are used for finding the border lengths. The diameters of the cells were measured by means of a horizontal microscope and the outline of the cells calculated.

The drawings of the cells in a unit area from the concave and convex sides of young and old tendrils of *Mukia scabrella*, Arn., are shown in Figs. 18, 19, 20, and 21.

For instance, the cell No. 5 in Fig. 20 is oval-shaped so its border length = $6 \times OP = 2.25$ cm. ($OP = 0.0375$ cm. by actual measurement). The value obtained is to be divided by 450, the magnification.

The following values are obtained for the border lengths of cells on the concave and convex sides in Figs. 18, 19, 20, and 21.

TABLE X.

Table showing Sums of the Perimeters of the Cells in Unit Area in Different Species.

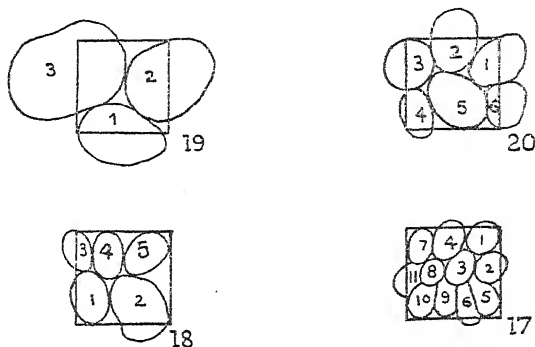
<i>Mukia scabrella</i> , Arn.		<i>Cucumis sativus</i> , L.	
	Concave. cm.	Convex. cm.	Concave. cm.
Uncurled	0.0184	0.0177	0.0271
Curled	0.0176	0.0109	0.0223
<i>Cucumis Melo</i> , L.		<i>Coccinia indica</i> , Wight and Arn.	
Uncurled	0.0299	0.0256	0.0352
Curled	0.0258	0.0192	0.0335
<i>Momordica Charantia</i> , L.		<i>Lagenaria vulgaris</i> , Seringe.	
Uncurled	0.0236	0.0217	0.0249
Curled	0.0226	0.0131	0.0251
<i>Cucurbita Pepo</i> , L.		<i>Luffa acutangula</i> , Roxb.	
Uncurled	0.0243	0.0223	0.0298
Curled	0.0251	0.0150	0.0297
<i>Benincasa cerifera</i> , Savi.		<i>Citrullus vulgaris</i> , Schrad.	
Uncurled	0.0245	0.0227	0.0268
Curled	0.0247	0.0175	0.0249
<i>Melothria perpusilla</i> , Cogniaux.		<i>Trichosanthes Anguina</i> , L.	
Uncurled	0.0267	0.0176	0.0252
Curled	0.0246	0.0178	0.0208
Young tendril (Figs. 19 and 20).		Old tendril (Figs. 17 and 18).	
Concave side	8.31 ÷ 450 = 0.0184 cm.		8 ÷ 450 = 0.0176 cm.
Convex side	7.9425 ÷ 450 = 0.0177 cm.		4.92 ÷ 450 = 0.0109 cm.

The results clearly show that the dense material is concentrated on the concave side which bears the strain of compression resulting from the curvature. In calculating the differences of the cell-wall lengths above given in Table X the thickness of the cells on the two sides is not taken into account. The cell-walls of the cells on the concave side are slightly thicker than those of the cells on the convex side, and that would also add to the mechanical strength of the cells on the concave side. The difference in the thickness is so slight that it is not possible to measure it accurately.

The greater density of the cell-wall material can also be shown by counting the number of cells in an unit area on the concave and convex sides. The cells of the convex sides are larger than those of the concave side and hence the number of cells on the convex side is less than the number of cells on the concave side in a unit area. The fewer the cells the larger the area of cell cavity and the less the cell-wall material in unit area, and vice versa.

On account of the position and nature of the parenchymatous cells and

their presence in the tendrils of all the plants investigated it was thought that curvature of the tendrils on contact was probably caused by the elongation of these cells on the convex side. These cells are mostly situated towards the convex side which should increase in length in order



FIGS. 17-20. Drawings of cells in an unit area from the concave and convex sides of young (Figs. 19 and 20) and old (Figs. 17 and 18) tendrils of *Munkia scrabrella*, Arn. $\times 450$.
For explanation see text.

that a tendril may curve. The cells are large and thin-walled and therefore are capable of elongation.

In order to prove the curvature is caused by elongation of these cells and is not due to growth by cell division it was necessary to show the actual elongation by measurements. It is evident on microscopical examination that the cells on the convex side do not divide after the stimulus of contact is received. There is complete absence of cell division in that tissue. It is also inconceivable that the cell division can take place in such a short time as the reaction times given above for different species indicate.

There are numerous difficulties in measuring the dimensions of the cells in the tendrils in uncurled and curled conditions. The measurements of the cells in an uncurled and curled condition cannot be made on the same tendril. So it is necessary to select two tendrils in the same stages of growth, otherwise the original dimensions of the cells of the two tendrils would be different and no comparison could justly be made. The age of the tendrils was judged from their length, and so the tendrils of the same length are very likely of the same age, as can be seen from Tables I to IV. Nearly full-grown tendrils should be selected.

The dimensions of the cells vary within wide limits. They also vary from the base of the tendril up to its apex. So it is necessary to measure the length of the cells of the tissue in these two conditions (curled and uncurled) at the same distances from the apices of the tendrils.

The third difficulty in making these measurements lies in keeping

the portion of the uncurled tendril in an uncurled condition during the operations of making measurements of the cells. The part of the tendril selected for making the measurements curled during the cutting of the sections; so the cells elongated before the measurements could be made. Sections could not be cut in water as they curled during the process. Various fixatives were tried. The pieces curled in chrom-acetic acid; the least curvature took place in formalin alcohol; they also curl in liquid paraffin. On account of this difficulty the differences in the dimensions of the cells in uncurled and curled conditions are understated.

Drawings of the cells were made under the camera lucida and the lengths of the different cells measured. All precautions to get the average dimensions of the cells were taken. A large number of cells of the different rows were measured from different longitudinal sections and the average dimensions of the cells of each row, beginning from the epidermis, were measured. The average length of the cells in each row was then found out both in curled and uncurled tendrils and compared.

In the Table XI the percentage increase in the cells of each row when the tendrils curl is given.

The figures represent in μ the average length of a cell in each row.

The measurements of the cells before and after elongation in the uncurled and the curled tendrils show a definite increase in length of the cells on the convex side. But the results are not uniform or regular as the elongations of the cells of different rows inside the epidermis vary within wide limits. The results do not indicate a gradual increase in the percentage elongation of the cells commencing from the centre towards the epidermis. The irregularities are due to the difficulties of measurement explained above, but the fact that the elongation occurs cannot be doubted.

It is also seen that the cells of different rows do not elongate in proportion to the physical requirements, when the curvature occurs; for this reason when the inner rows of cells elongate more than the outer rows including the epidermis, folds are seen on the convex sides. The cells of the epidermis and the two or three inner rows are drawn in at some points on the convex surface due to vigorous elongation of the cells in the inner rows. Such foldings on the convex side are noticed in *Cucumis sativus*, L., *Cucumis Melo*, L., *Momordica Charantia*, L., *Mukia scabrella*, Arn., *Cucurbita Pepo*, L., and *Lagenaria vulgaris*, Seringe.

The following reasons may be given to support the view that the elongation of the cells on the convex sides is responsible for the curvature of the tendrils in Cucurbitaceae.

1. The observed lengths of the cells in the uncurled and curled condition.

TABLE XI.

Momordica Charantia, L.

	Epidermis	Cells of the 1st row.	2nd.	3rd.	4th.	5th.	Total.	Average.
Curled	55.54	68.88	76.59	107.17	106.56	99.98	514.72	85.786
Uncurled	35.54	42.22	42.22	53.32	73.32	97.76	344.38	57.396
Increase in length	20.00	26.66	34.37	53.85	33.34	2.22	170.34	28.390
% elongation	56.34	63.03	81.5	100.94	45.3	2.25	49.44	49.47

Mukia scabrella, Arn.

Curled	32.56	68.82	109.94	170.94	166.5	81.03	629.79	104.96
Uncurled	19.98	43.41	46.06	52.17	74.37	64.38	291.37	48.56
Increase in length	12.58	34.41	63.88	118.77	92.65	16.65	338.42	56.40
% elongation	62.58	100	138.7	225.9	123.9	25.86	116.1	116

Coccinia indica, Wight and Arn.

Curled	31.1	74.37	66.66	99.98	84.42		356.53	71.30
Uncurled	24.44	54.76	44.44	64.44	75.54		263.62	52.72
Increase in length	6.66	19.61	22.22	35.54	8.88		92.91	18.58
% elongation	27.26	35.81	50	55.14	11.76		35.24	35.25

Cucurbita Pepo, L.

curled	22.83	150.96	115.44	82.14	79.98	155.40	606.75	101.12
Uncurled	18.59	84.36	59.94	46.66	50.20	91.10	359.85	59.80
Increase in length	4.24	66.60	65.50	35.48	20.78	64.30	256.90	41.32
% elongation	22.81	78.96	109.2	76	35.11	70.58	71.30	69.1

Cucumis sativus, L.

Curled	33.3	137.64	70.98	76.96	166.5	134.88	620.26	103.37
Uncurled	15.31	46.62	39.40	75.48	68.82	74.37	320.00	53.33
Increase in length	17.99	91.02	31.58	1.48	97.68	60.51	300.26	50.04
% elongation	117.5	125.2	95.61	1.961	141.9	81.37	93.83	93.85

Cucumis Melo, L.

Curled	37.18	42.18	79.92	67.34	63.64		290.26	58.05
Uncurled	20.22	28.86	39.07	47.36	62.16		197.67	39.53
Increase in length	16.96	13.32	40.85	19.98	1.48		92.59	18.5
% elongation	83.89	46.14	104.6	42.2	2.38		46.85	46.85

Lagenaria vulgaris, Seringe.

Curled	36.15	123.22	168.72	142.08	279.76	208.68	958.61	159.77
Uncurled	7.77	51.10	33.32	63.64	66.66	69.93	292.42	48.74
Increase in length	28.38	72.11	135.40	78.44	213.10	138.75	666.19	111.03
% elongation	365.3	141.1	406.3	123.2	319.6	198.4	227.8	227.7

2. The nature and the character of the cells on the two sides of the tendrils.

3. The position of the cells. The extensible material is disposed in such a manner that the maximum elongation can take place.

4. The dense material is on the concave side to withstand the pressure of compression, when the other side elongates. The cells on the concave side show the effect of pressure as the transverse walls are thrown into folds and project into the cavities of the cells.

5. There is no contraction of the cells on the concave side as the measurements showed. The infolding of the transverse cell-walls is not caused by contraction, but is the result of the pressure exerted by the elongating cells.

The following considerations are against the view that the curvature of the tendril takes place by unequal growth.

1. The reaction time is too short for growth to take place.

2. No cell division taking place after the stimulus of the contact is to be observed. It is not likely that cell division would occur and give rise to curvature within 30 to 60 seconds.

3. The tendency of the tendril to curve after it is placed in the fixatives, and liquid paraffin.

The question arises as to the causes which make the cells elongate on the reception of a stimulus. Is the elongation due to growth in the surface of the cells, if it is not due to growth by cell division? It cannot be the growth in surface of the cells as the time is too short. It is probably physical extension of the cell-walls. The elongation of the cells is not solely caused by the increase in turgidity of the cells as was suggested by de Vries (2). If so a curled tendril could be made to uncurl by putting it in a strong solution of an osmotically active substance and removing the turgidity. This has not been found possible, as curled tendrils do not straighten out in a strong solution of sugar or common salt.

The following view of the mechanism responsible for the rapid curvature of the tendrils of Cucurbitaceae is a deduction from the physiological and anatomical facts, brought out in this investigation. A fully grown tendril is in a state of internal tension on account of the tendency of the cells on the convex side to elongate. The tendency to elongate is confined to the convex side (abaxial side) only, and hence the uniform elongation in length of the tendril is not possible. Here one side of the bilateral tendril tends to become longer than the other, and this should give rise to curvatures. But the curvature is prevented by the resistance offered by the adaxial (concave) side on account of the dense material concentrated on the concave side. Naturally the whole tendril is in a state of tension, one side of the tendril trying to elongate and the other side of the tendril offering resistance against bending and withstanding the

pressure exerted by the other side. In an uncurled fully grown tendril a temporary state of equilibrium between the two opposite forces is obtained.

The state of tension begins after the tendril is about one-third grown, it increases as the tendril becomes mature, and it reaches the maximum when it is fully grown. The state of tension is correlated to the degree of sensitivity of a tendril, the state of greatest tension being the stage when the tendril becomes the most sensitive.

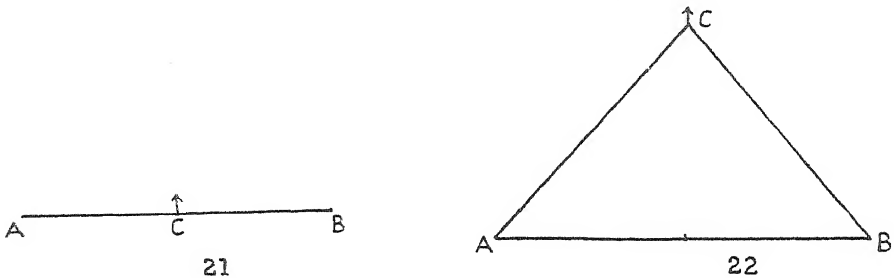
When a fully grown tendril is cut up into small pieces about 2 cms. long an escape of drops of water is noticed from the cut ends of the pieces. If these pieces are kept between moist pieces of paper they show slight curvatures, but if they are placed in water the curvature takes place rapidly and it is very pronounced. These experiments suggest that the curvature of tendril takes place when the tension is released and that water plays a part.

Thus an uncurled tendril on the plant is in a state of tension internally, and it can only curl when there is a slight fall in the tension on account of some disturbance in its equilibrium. The disturbance probably results when the tendril rubs against an uneven surface, or touches a support when the internal tension is released at that point. It is not understood how the fall in tension can occur when the tendril rubs against an uneven surface. The fall in tension is probably followed up by the escape of water from the conducting elements into the cells which undergo elongation. This can be assumed from the escape of water from the cut ends of the pieces of tendril and curling of the pieces in water. The fall in the tension, caused by an external force, acts as a liberating factor for the passage of water into those cells which begin to elongate. The concave side of the tendril is passively bent under the pressure of the elongating side. The fall in tension at one point is carried to the other portions of the tendril in the following manner.

Imagine a point C where the disturbance is produced and two other points A and B (Fig. 21) situated at equal distance from C on the tendril. When the point C comes in contact with a support there is a fall in the tension which causes a disturbance in the interior of the tendril. The disturbance caused by the fall in the tension is maximum at C, and it falls off as we go farther and farther from C towards A and B. At A and B, which are supposed to be at sufficiently large distance from C, no disturbance is felt.

When the fall in the internal tension of the tendril caused by the disturbance occurs there is a natural tendency in the tendril to be displaced on the upper side of ACB. The displacement at C causes fresh disturbance and brings about a further fall in the tension. The disturbance now reaches the points A and B, and the points of the tendril beyond them as the

displacement increases. As the angle to the normal increases in (Fig. 22) the tension in the tendril becomes less and less till it disappears completely at C.



FIGS. 21 and 22. For explanation see the text.

The same can be said with regard to other points on the tendril. In this way in the older parts of the tendril *not* in contact with the support the fall in the internal tension occurs in the manner explained above, and the free portion of the tendril is thrown into spirals due to the elongation of the cells on the convex side.

SUMMARY.

1. The daily growth in length of the tendrils of some species of Cucurbitaceae has been measured. The growth in length is confined to the apical region in the tendrils of some of the species, while in others the basal region grows in length more vigorously than the apical.

2. The reaction time to the stimulus of contact varies in the tendrils of different species, and also depends on the age of a tendril. The sensitivity increases as the tendrils become mature. The time taken to form one complete coil after the application of the stimulus depends upon the age of the tendrils, as does also the number of coils formed round a support. The number of coils round a support is greater in young tendrils than in mature ones, indicating that the tendrils grow in length even after they reach a support.

3. The anatomy of the tendrils is investigated, and it is shown that the cell-wall material is more dense on the concave side than on the convex side. The parenchymatous cells on the convex sides of the tendrils elongate and give rise to curvatures. Measurements are made of the cells on the convex sides before and after curvatures.

4. An attempt is made to explain the mechanism of curvature of tendrils in the Cucurbitaceae.

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The Question of Golgi Bodies in the Higher Fungi.

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With eleven Figures in the Text.

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I. INTRODUCTION.

THE question of Golgi bodies in plant tissues has only recently been investigated. In 1922 Guilliermond and Mangelot of Paris obtained Golgi apparatus in barley roots by the silver-impregnation method. In March 1926, Guilliermond published a paper (12) in which he discussed the relation between the plant-vacuolar system and the Golgi apparatus. He treated the epidermal cells of very young leaves of *Iris germanica* and meristematic tissues of young shoots of *Elodea canadensis*, some *Chlorophyceae*, *Cyanophyceae*, *Bacteria*, and some fungi (*Yeast* and *Oidium lactis*) with the silver-impregnation methods of Cajal and da Fano as well as with vital staining with neutral red. In the majority of cases he was able to obtain the precipitates of vacuoles (metachromatic corpuscles) stained in the form of the network of canals constituting the Golgi apparatus. Botanists generally are of opinion that the plant-vacuolar system and the Golgi canals of animal-cells are morphologically and physiologically equivalent. This is supported by the remarkable researches of Parat (20), who has shown that the Golgi apparatus also in animal-cells consists of a number of

vacuoles. Dr. D. R. Bhattacharya (1) of Allahabad, India, working on a number of Vertebrates, holds that 'the Golgi bodies and vacuoles (vacuome) are homologous structures'. The recent conclusions (in 1928-9) of Vishwa Nath of the Panjab University (17, 18, 19), as a result of his cytological study of the oogenesis of *Crossopriza* and *Scolopendra* and of the egg follicle of *Culex fatigans*, are that 'the Golgi elements exist in the form of vacuoles with watery and non-fatty contents, that the solid Golgi elements are artifacts due to the excessive precipitation of metallic silver or osmium inside the vacuoles, and that the Golgi crescents are either artifacts due to the incomplete blackening of a vacuole or they are simply the optical sections of an osmicated vacuole'. Of the present-day cytologists Prof. C. E. Walker, of Liverpool University, seems to have paid well-merited attention to the presence of artifacts in fixed materials; he has shown that 'the Golgi bodies can be produced artificially by the same treatment that is necessary to make them apparent in fixed tissue cells', hence it may be 'that the appearance of Golgi bodies may be due entirely to the action of the reagents used upon the homogeneous cytoplasm, and that (perhaps) no structures of the kind exist in the living cell' (23). Further, Walker (24) has given an account of the conditions in which his *artificial* Golgi bodies may be made to take up a constant position in relation to the nuclei in his mixtures—conditions which may well be produced by the nucleus of the living cell in certain circumstances.

Recently, Bowen in a number of papers has described some small rod-shaped structures with osmiophilic cortex and osmiophobic medulla in members of the *Spermatophyta*, *Pteridophyta*, and *Bryophyta*. These he calls 'osmiophilic platelets', and regards them as the homologues of the Golgi bodies of animal-cells. The existence of these platelets can be demonstrated in plant-cells only by the Kolatchev and Mann-Kopsch (Weigel) methods, but they are not visible by the methods of Bouin, Benda, Fleming-without-acetic, or with the classical silver-nitrate method of Golgi; neither can they be brought out by vital staining with neutral red. Gatenby (10), following the methods of Bowen in fresh root-tips of bean, hyacinth, and in pea-shoots, has confirmed the presence of 'osmiophilic platelets' in plant-cells, and agrees with the interpretation of Bowen that these platelets probably represent the Golgi elements in the plant-cell. 'So far, no work of this kind seems to have been done on higher fungi (vide Cowdry); Bowen states that he has not worked on Thallophyta.

II. MATERIAL AND METHODS.

Material. A number of our common *Polypores* and *Agarics* were collected fresh with the sporophore, viz. (1) *Polyporus zonalis*, (2) *P. Ostreiformis*, (3) *Gandoderma* (*Fomes*) *lucidum*, (4) *G.* (*Fomes*) *colossus*,

- (5) *Lenzites repanda*, (6) *Lentinus subnudus*, (7) *Schizophyllum commune*, (8) *Lepiota mastoideus*, (9) *Flammula dilepis*, (10) *Entoloma microcarpum*, (11) *Psathyra lucipeta*, (12) *Panacolus cyanascens*.

Small pieces of the hymenial surface were at once fixed; in some cases it was necessary to use an air-pump to make the bits sink in the fixing fluid.



FIG. 1.

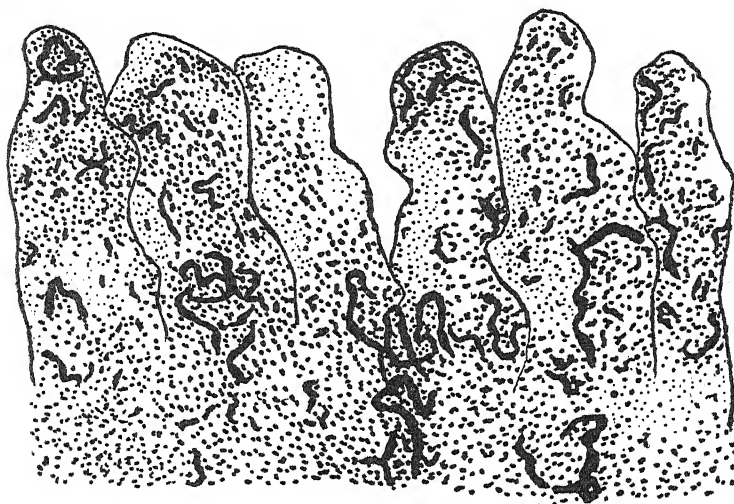


FIG. 2.

FIGS. 1, 2. Camera lucida drawings under Zeiss apochromat 2 mm. N.A. 1.4, with compensating ocular No 15. 1. Basidia of *Lentinus subnudus*, Golgi's silver-nitrate method. $\times 1,875$. 2. Basidia of *L. subnudus*, Golgi's silver-nitrate method. $\times 1,875$.

For comparison with Bowen's results, root-tips of germinating seedlings of pea and bean were fixed in Champy's fluid.

Methods. The ordinary solutions of Fleming without acetic and of Bensley (neutral formalin and potassium bichromate) were tried, as well as Golgi's bichromate and silver-nitrate method (rapid process), and Kolatchev's method as described in detail by Bowen (5). The Weigl method was also given a short trial, but not being so satisfactory as Kolatchev's it was discontinued.

In following Golgi's method three parts of 3 per cent. potassium bichromate and one part of 1 per cent. osmic acid were used to fix small pieces of fresh hymenial tissues, and the silver-impregnation was always conducted

in the dark from three to four days within an incubator at 35° C. By trial experiments it was found that the specimens hardened in potassium bichromate and osmic acid from six to ten days showed good impregnation

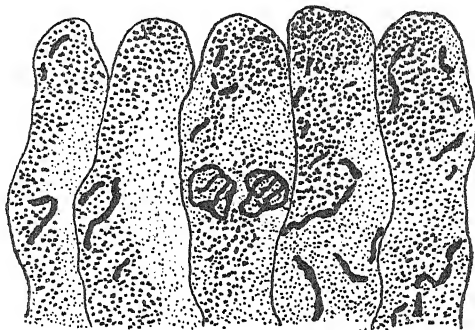


FIG. 3.

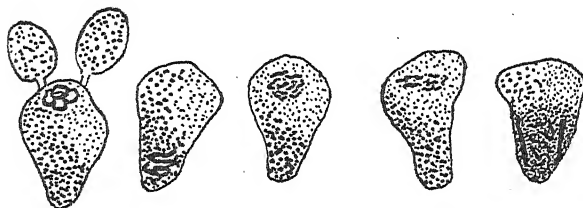


FIG. 4

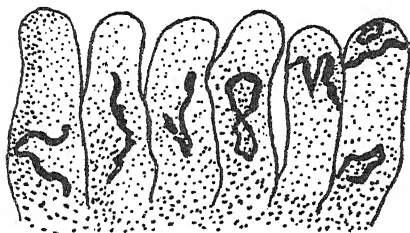


FIG. 5.

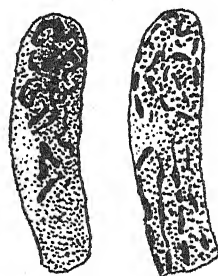


FIG. 6.

FIGS. 3-6. Camera lucida drawings under Zeiss apochromat 2 mm. N.A. 1.4, with compensating ocular No. 15. 3. Basidia of *Schizophyllum commune*, Golgi's silver-nitrate method. $\times 1,875$. 4. Basidia of *Panaeolus cyanascens*, Golgi's silver-nitrate method. $\times 1,110$. 5. Basidia of *Polyporus ostretiformis*, Golgi's silver-nitrate method. $\times 1,875$. 6. Basidia of *Lenzites repanda*, Golgi's silver-nitrate method. $\times 1,875$.

and formation of coils within the basidia, resembling the Golgi apparatus of animal-cells (Figs. 1 to 6). The favourable conditions for the silver-impregnation usually declined after the tenth day, in some cases even earlier. Finally, thin sections were cut free hand without embedding in paraffin, and mounted in *thick cedar-wood oil* under a cover-slip after clearing in *fluid cedar-wood oil* for an hour. Paraffin sections were rather poor as found by Golgi, and therefore the paraffin method was given up.

In trying the Kolatchev method the specimens, after fixing in Champy's fluid and thorough washing in running water for twenty-four hours, were taken from distilled water to 2 per cent. osmic acid and incubated at 40° C. for about twelve hours; they were then kept in the dark at the room temperature (which was about 35° C. at the time) for the proper grade of osmication. By successive trials it was found that the optimum osmication varied from the seventh to the tenth day. After the usual procedure paraffin-sections were cut 5 μ with the microtome. Bleaching was carried out by the hydrogen-peroxide (quick) method, the action being carefully watched under the microscope.

Control method of vital staining with neutral red: a 0.001 per cent. watery solution of neutral red was employed in the case of sections of the hymenia of fresh specimens. The red stain of the metachromatic corpuscles within the vacuoles came out in about fifteen minutes. Of course, the colour depends on the nature of the colloidal contents of the vacuoles, and is determined, according to Guilliermond (14), by the flocculation of these colloids. The use of concentrated solution causes the dissolution of the precipitated substances, leading to diffuse coloration or very faint coloration of the vacuoles. The stain is strictly limited to the colloids within the vacuoles.

III. OBSERVATIONS.

In the course of my extended cytological investigations of the basidia of a number of species of *Polyporus* and *Agaricus* with Rigaud's fluid and Fleming- (both strong and weak) without-acetic, I have come across only two classes of substances in the general cytoplasm-vacuoles and mitochondria. The mitochondria consist of small granular bodies called chondriosomes or sphaerosomes, and of very small rod-shaped structures called 'petits batonnets' by Guilliermond; long filamentous mitochondria (such as are known as plasts in higher plant-cells) are hardly found (Fig. 7). In Bensley's fluid the system of vacuoles within the basidia came out prominently as a unit; it was by no means uniform, depending as it did on the stage of development of the basidia.

By the silver-impregnation method the basidia of the majority of the specimens showed the formation of blackened coils, sometimes united into a net in varying positions, in some cases close to the top of the basidia, in others at the bottom, in some, again, on the sides almost at the central part of the basidia (Figs. 1 to 6). By means of the vital staining with neutral red it could be seen that these black coils usually corresponded with the positions of the vacuoles within the basidia. These coils are nothing but artifacts due to the precipitation of metallic silver inside the vacuoles, the metachromatic bodies within the vacuoles taking the stain; thus, when a number of vacuoles run together, they give rise to a net-like appearance or 'curved moniliform rods', as has been described and figured by Vishwa Nath

(19, p. 660). The results so far obtained confirm the experience of a number of workers (Guilliermond, Vishwa Nath, &c.) that the silver-nitrate preparation is by no means a constant and steady one.

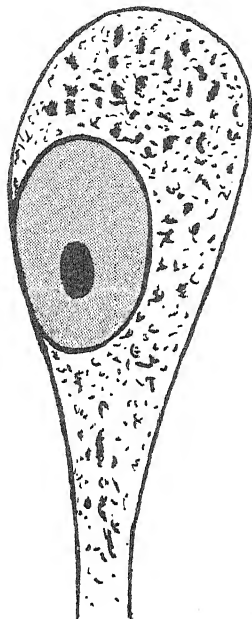


FIG. 7. Camera lucida drawing under Zeiss apochromat 2 mm. N.A. 1.4. Basidia of *Polyporus squamosus*, Rigaud's method. $\times 5,000$. For this drawing the tubelength of the microscope was increased.

I have already reported (2) that, employing the silver-nitrate method of Golgi, I obtained in the root-tips of *Allium*, *Lilium*, and *Canna* clear blackened networks (in the form of the Golgi bodies of Gatenby) in close association with nuclei.

With the Kolatchev method good differentiation of the vacuome within the basidia of higher fungi was obtained; the basidia showed only a number of round vesicular bodies located in different parts—some towards the top, others towards the base or central region, while some were entirely filled with them all through their lengths (Figs. 8, 9, 10). The nuclear membrane apparently had been broken down artificially in many of the paraffin-sections cut 4 or 5 μ , hence the nucleus was not visible (Cf. Vishwa Nath, 19, p. 668). In some only the membrane could be found (Fig. 11). A good many of these spherical bodies have a distinct black chromophilic rim and a central chromophobic area, while some appear entirely black and solid on account of the excessive precipitation of osmium inside their interior. These are mostly the metachromatic corpuscles within the vacuoles, as can be verified by the control experiment of vital staining with neutral red, when they appear almost in the same position within the basidia.

Some of the vesicular bodies within the basidia might be formed by the action of the fixative on the mitochondria, as has been remarked by Guilliermond (12, p. 14, Fig. 4 F). Nowhere could I find within the basidia the rod-shaped structures called 'osmiophilic platelets' by Bowen (4), and regarded by him as equivalent to the Golgi bodies of animal cells. Bowen, on examining some of my slides (Kolatchev preparations) sent to him, states in his letter as late as 29th January, 1929, that 'the blackened bodies in your slides differ quite decidedly from my osmiophilic platelets. . . . The blackened bodies in your preparation of fungi are probably not what I have identified as osmiophilic platelets in higher plants. . . . In the case of blackened bodies in your preparations, however, it seems to be a case of vesicular formation the periphery of which is blackened'. One of my Kolatchev preparations of fungi was sent to Prof. Guilliermond, of Paris, who, after examining the slide, remarks in his letter dated 28th

October, 1928, 'Je suis entièrement de votre avis, les corps vesiculaires que vous avez obtenu dans les polypores ne sont autre chose que les corpuscules métachromatiques produits par précipitation d'une substance se trouvant à

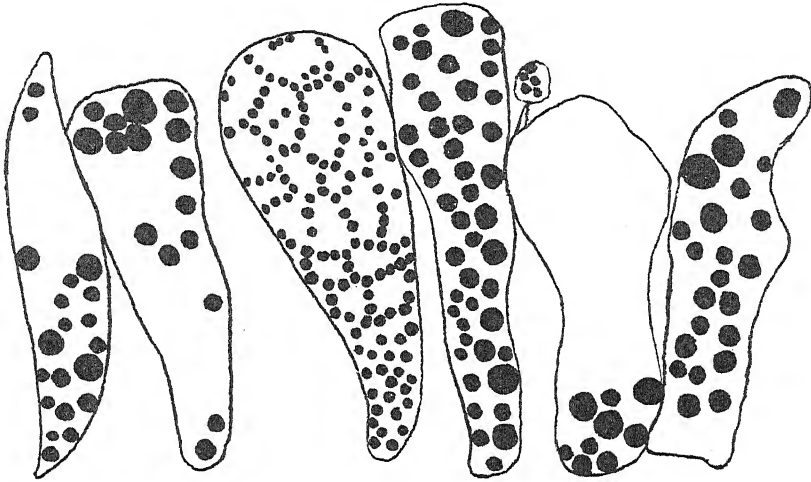


FIG. 8.

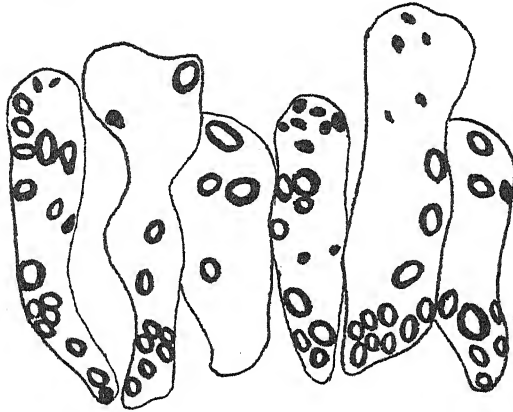


FIG. 9.

FIGS. 8, 9. Camera lucida drawings under Zeiss apochromat 2 mm. N.A. 1.4, with compensating ocular No. 20. 8. Basidia of *Entoloma microcarpum*, Kolat. method. $\times 2,500$. 9. Basidia of *Polyporus ostreiformis*, Kolat. method. $\times 2,500$.

l'état de solution colloïdal dans les vacuoles. J'ai obtenu les mêmes formations dans divers champignons'.

Staining *intra vitam* with the dilute aqueous solution of neutral red (.001 per cent.), the small metachromatic bodies within the vacuoles took a deep red stain in the form of fine droplets; big round drops taking a diffused stain showed in their interior a number of stained bodies; these are the vacuoles found in varying positions within the basidia.

All these results confirm my observation (2) referred to above, that the vacuolar bodies in plant-cells probably represent the Golgi bodies of animal-cells.

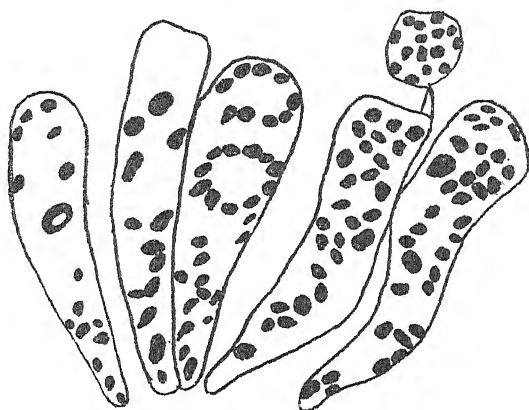


FIG. 10.

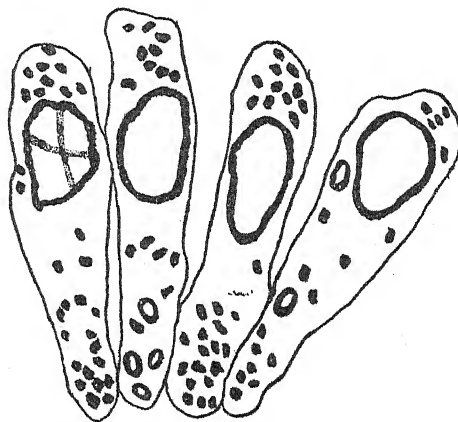


FIG. 11.

FIGS. 10, 11. Camera lucida drawings under Zeiss apochromat 2 mm. N.A. 1.4, with compensating ocular No. 20. 10. Basidia of *P. ostreiformis*, Kolat. method. $\times 2,500$. 11. Basidia of *P. ostreiformis* showing nuclear membrane, Kolat. method. $\times 2,500$.

IV. DISCUSSION.

Bowen (4, 5) regards 'osmiophilic platelets' as the fourth type of cell-constituents in plants besides plastidome, pseudochondriome, and vacuome, while Gatenby's (10) rather short experience of plant-cells has led him to conclude that there exist only three systems in plant-cells, namely chondrisome, plastidome, and 'osmiophilic platelets' (Golgi elements); he summarily dismisses the vacuome from the category of the permanent cytoplasmic elements in plant-cells.

I have repeated Bowen's work on root-tips of pea and bean, following

the Kolatchev method in detail, and I was able to find those platelets exactly as he has figured. To me they appear to be nothing but distorted mitosomes and fragmented *discoplasts* of Dangeard (or 'petits batonnets' of Guilliermond), common in all plant-cells and demonstrated by the ordinary methods of Regaud or Fleming-without-acetic. Here they are certainly greatly altered and have undergone partial vesiculation forming a chromophilic rim and a chromophobic area due to the irregular and capricious action of the osmic acid on plant cells (Guilliermond (13), pp. 369-70). Bowen himself admits (6, p. 35) that 'the osmic methods are so tedious, so variable, and so often unsuccessful'. Vishwa Nath, in his recent paper in March 1929 (19, p. 660), remarks that 'in spite of the brilliant results that osmic acid and silver nitrate gave in the hands of skilled workers these methods are admittedly capricious'. It does not seem safe, therefore, to base a *discovery* on a method which is admittedly so variable and capricious, especially when there is no control experiment of vital staining of living protoplasm. Bowen (7, p. 32) argues that his platelets are of a constant type and hence cannot be artifacts from elements of varying morphology. Walker (23, 24) has, however, shown that this constancy is no ground for believing structures to be real components of the living protoplasm.

Both Bowen and Gatenby admit that the osmiophilic platelets (Golgi elements of plants) are demonstrable in plant-cells only by the special methods of Kolatchev and Weigel, but they are not found when treated with the methods of Benda, Fleming-without-acetic, or the silver nitrate method of Golgi. This raises a strong presumption against their being a universal constituent of the living plant-protoplasm. A structure that has to be invariably a component of the cytoplasm of *all* plant-cells ought to come out with the ordinary cytological methods.

Bowen, while attempting in a series of papers a complete and exhaustive survey of the structural elements of plant-protoplasm, curiously leaves out of account the very small rod-shaped structures (called mitosomes or 'petits batonnets' of Guilliermond) found in all plant-cells. His pseudochondriomes—'typically small spheres', as he calls them, in Ann. Bot. vol. xliii, 1929—correspond to the spherosomes of Dangeard, but nowhere does he mention any structure corresponding to the 'mitosomes' of Dangeard. As I have said before, a 'mitosome' can easily look like a hollow rod with a black rim due to the action of the osmic acid in the fixative. It is not, therefore, a matter of surprise that Bowen has mistaken these distorted mitosomes for his 'osmiophilic platelets'.

Regarding the vacuolar system, Gatenby (10) says that 'no such system has been demonstrated in plant-cells, there is no evidence that the vacuoles are formed from self-perpetuating primordia'; but a few months later (11) he mentions in the concluding part that 'the plant cell contains *perhaps* a vacuolar system'. In this connexion I would draw

attention to the conclusions of Dangeard and Guilliermond (Sharp (22), pp. 44-5) that 'the vacuome (vacuolar system) is a permanent constituent of the cell, that it is an autonomous system present in all plant cells in the form of minute metachromes, recognizable vacuolar material, or aleurone grains, that by repeated division it is passed on from cell to cell and from generation to generation'. In 1927 Eftimiu and Kharbush (8, p. 85) observed clearly the passage of the vacuoles from the basidia through the sterigmata to the basidio-spore of *Exobasidium*, the germinating spores pass on the vacuoles in their turn to the germ tubes, and from the tubes they are passed on to the mycelium. From these observations they conclude that the vacuoles are passed on from cell to cell fully conserving their individuality, and that they cannot be explained as arising *de novo*. The very recent paper of Guilliermond (15) on 'the recent development of our idea of the vacuome of plant-cells' also shows conclusively that the vacuome is present in every plant-cell.

Bowen (4) observes that 'it appears probable that these bodies ('osmiophilic platelets') as a permanent and generalized component of plant-cytoplasm have never been described before, indeed may very probably *never have been seen* by botanists. It is an extraordinary fact that Guilliermond does not mention these platelets in his recent papers.' Assuredly, the French school of plant-cytology, founded by Dangeard and Guilliermond, can claim a long and wide experience of plant-cells. In the present state of our limited knowledge of cell-structure it would be very valuable to follow side by side both the methods of animal and plant cytological technique in the study of plant-cells and at the same time to compare with the living protoplasm using vital staining. Professor Walker, in a private communication (1929) has rightly remarked that 'artifacts are probably the most dangerous pitfalls for the cytologists'.

It is curious that Gatenby was quite unsuccessful in staining the vacuome of plant cells by neutral red, and that Bowen uniformly failed to obtain any result with the well-known method of Regaud in the study of plant-cells.

Both Bowen and Gatenby have attempted to establish a generalization covering both animal and plant cells. In the *Basidiomycetes*, however, I have already observed that by the Kolatchev method no 'platelets' are found within the basidia; this is admitted by Bowen in his letter quoted above. In my opinion, therefore, this generalization regarding the presence of the Golgi elements ('osmiophilic platelets') in all plant-cells fails so far as the higher fungi are concerned.

Finally, may I point out the confusion created by Gatenby regarding Mottier's suggestions which he (Gatenby) has mistakenly transposed (10, pp. 399-400)? A reference to Bowen's summary in his recent paper (7, p. 324, third and fourth paragraphs) will at once show that Bowen fully

agrees with the findings of Mottier. Recently, Parat (21) has shown that 'Dictyosomes' are nothing but chondriosomes pachynetique (mitochondrial in origin).

V. SUMMARY AND CONCLUSIONS.

The results so far obtained confirm the author's observation (2) that the vacuolar bodies in plant-cells probably represent the Golgi bodies of animal-cells.

The blackened coils within the basidia of higher fungi are nothing but artifacts due to the precipitations of metallic silver in the vacuoles, the running together of a number of vacuoles giving rise to a net-like appearance or to 'curved moniliform-rods', as described and figured by Vishwa Nath. This has been confirmed in a number of animals recently investigated by Vishwa Nath and Bhattacharya, where the Golgi elements as reported by them are in the form of vacuoles, the solid Golgi elements being artifacts due to the excessive precipitation of metallic silver or osmium inside the vacuoles.

It is suggested that the 'osmiophilic platelets' (Golgi elements) of Bowen are nothing but distorted mitosomes or 'petit batonnets' of Guilliermond, greatly changed owing to the irregular action of the osmic acid on plant-cells.

With the Kolatchev method not a single rod-shaped structure, i.e. 'osmiophilic platelet' of Bowen, is found within the basidia of the higher fungi; the basidia show only a number of round vesicular bodies—the metachromatic corpuscles within the vacuoles. The premature generalization regarding the presence of the Golgi elements in *all* plant-cells cannot therefore hold.

The recent works of Eftimiu and Kharbush on *Exobasidium* show conclusively that the vacuolar system is a permanent constituent of the plant-cell, that it is passed on by repeated division from cell to cell and from generation to generation, and that it never arises *de novo*. The previous works of Dangeard and Guilliermond also point to the same conclusion. Further, the very recent paper of Guilliermond (15) gives a fairly complete knowledge of the vacuome in plant-cells.

The fact that the 'osmiophilic platelets' are demonstrable in plant-cells only by the special methods of Kolatchev and Weigel raises a strong presumption against their being a universal constituent of the living protoplasm.

In the present state of our limited knowledge of cell-structures the methods both of animal and plant cytology ought to be followed side by side in the study of plant-cells, and at the same time the results should be compared with those obtained by vital staining with neutral red.

ADDENDUM.

Since the above was written Bhattacharya and Vishwa Nath have changed their views (*Nature*, Nov. 2, 1929, and *Quart. Journ. Micros. Sci.*, lxxiii, Pt. III, 1930). They now hold that in *animal* cells the vacuoles are distinct from the Golgi bodies (Feb. 1931).

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The Oidia of *Coprinus lagopus* and their Relation with Insects.

BY

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With Plate X and twenty-four Figures in the Text.

I. INTRODUCTION.

THE researches of Eidam, van Tieghem, Brefeld, Zopf, and others have taught us that, in many Hymenomycetes and in certain Pyrenomycetes and Discomycetes, the young mycelium developed from a basidiospore or from an ascospore produces or breaks up into a series of short segments which Brefeld called *oidia*. Brefeld (2, 3) in his *Untersuchungen* illustrated the oidia of the following Hymenomycetes:

<i>Coprinus lagopus</i>	<i>Hypholoma fasciculare</i>
<i>Galera tenera</i>	<i>Pleurotus ostreatus</i>
<i>Panacolus campanulatus</i>	<i>Collybia velutipes</i>
<i>Stropharia semiglobata</i>	<i>C. maculata</i>
<i>S. melasperma</i>	<i>C. conigena</i>
<i>Schizophyllum lobatum</i>	<i>Pholiota marginata</i>
<i>Lenzites abietina</i>	<i>Psathyra spadiceo-grisea</i>
<i>Daedalea unicolor</i>	<i>P. nolitangere</i>
<i>Trametes odorata</i>	<i>Psilocybe spadicea</i>
<i>Polyporus suaveolens</i>	<i>P. semilanceata</i>
<i>P. serialis</i>	<i>Clitocybe metachroa</i>
<i>P. zonatus</i>	<i>Nyctalis asterophora</i>
<i>P. versicolor</i>	<i>N. parasitica</i>
<i>P. quercinus</i>	<i>Naucoria scmiorbicularis</i>
<i>Phlebia radiata</i>	<i>Typhula variabilis</i>
<i>Irpex obliquus</i>	<i>Radulum laetum</i>

Brefeld's illustrations represent the oidia as short hyaline rod-shaped cells with thin cell-walls and dense protoplasmic contents.

In the nineteenth century, when mycologists were searching for sexual organs in the higher fungi, it was believed by Eidam (8) and van Tieghem (18) that oidia are male cells or spermatia, even although female organs

could not be found. Van Tieghem (19), in 1875, succeeded in germinating the oidia of *Coprinus plicatilis* and *C. stercorearius*,¹ and he then declared that the oidia serve to reproduce the fungus in an asexual manner and that, in this respect, they are comparable with conidia.

Brefeld (2), in 1877, announced that he had been unable to germinate the oidia of *C. lagopus*, although he had made numerous attempts to do so, and that he had come to the conclusion that oidia are vestigial structures which no longer possess the power of germinating, and which, therefore, cannot be regarded as functional spermatia. His observations that the fruit-bodies of certain Coprini owe their origin to the development of a single hypha of the mycelium proved that the co-operation of the oidia in the formation of fruit-bodies is unnecessary.

Falck (9), in 1902, described and illustrated the oidia of *Mucor racemosus*, *Dacryomyces deliquescens*, *Ascobolus lignatilis*, *Phlebia merismoides*, *Agaricus coprophilus*, *Chalymotta campanulata*, *Coprinus ephemerus*, *Hypholoma fasciculare*, *Collybia velutipes*, *C. tuberosa*, and *Oidium lactis*.

Falck succeeded in germinating the oidia of the wood-destroying fungi *P. merismoides*, *H. fasciculare*, and *C. velutipes*. Then, using a single oidium to inoculate his culture medium, with each of these species he succeeded in obtaining perfect fruit-bodies. We now know that, in general, oidia are produced only on haploid mycelia and not on diploid. It is, therefore, possible that Falck's fruit-bodies were all haploid and that the spores produced by each of them were of one and the same sex.

In 1909, Falck (10) showed that the oidia of *Leucisites sepiaria*, after having been kept dry for a year, are still able to germinate.

So far as coprophilous Hymenomycetes are concerned, Falck did not succeed in germinating the oidia of *C. lagopus*, *Panacolus campanulatus*, and *A. coprophilus*; and he discovered that *C. sterquilinus*, like Brefeld's *C. stercorearius*, fails to produce any oidia whatsoever. Falck (9) came to the conclusion that the oidia of most of the coprophilous Basidiomycetes have lost the ability to germinate, and therefore have nothing to do with the dissemination of these fungi.

In 1918, Bensaude (1), in her well-known paper on the life-history and sexual phenomena of *C. fimetarius*,² incidentally described and illustrated the structure and mode of production of the oidia. She saw some of the oidia germinate and produce short germ-tubes, which soon fused with cells of the parent mycelium.

¹ Doubtless van Tieghem misidentified this species, as the true *C. stercorearius* which develops sclerotia does not produce any oidia.

² Professor Buller has informed me that, after personal consultation with Mlle Bensaude, he came to the conclusion that her *C. fimetarius* and the *C. lagopus* described in his *Researches on Fungi* (vol. iii) are identical species.

Bensaude planted two mycelia of opposite sex, *A* and *B*, near to one another on nutrient agar, and she observed that occasionally one of the mycelia became diploid *before it had come into contact with the other one*.

Supposing that the mycelium which became diploid was *A*, she explained the transformation as follows: oidia from the mycelium *B* floated across the gap between *A* and *B* in the surface film of water covering the agar and then germinated; their germ-tubes fused with the mycelium *A*; and thus the mycelium *A* was converted by the oidia from the haploid to the diploid phase.

In 1927, Craigie (7), acting on a suggestion given to him by Professor A. H. R. Buller, discovered that the pycniospores of the Rust Fungi which are produced by haploid mycelia are functional. When (+) pycniospores are carried from a (+) pustule (in the laboratory by hand or under natural conditions by insects) to a (-) pycnium in a (-) pustule, the mycelium in the (-) pustule becomes diploid and produces diploid aecia and aeciospores; and, conversely, when (-) pycniospores are carried from a (-) pustule to a (+) pycnium in a (+) pustule, the mycelium in the (+) pustule becomes diploid and produces diploid aecia and aeciospores.

When Craigie's investigations on the Rust Fungi were in progress, Professor Buller conceived the idea that the oidia of the Hymenomycetes might function in a similar way to the pycniospores of the Rust Fungi, i.e. that (+) oidia might be carried, in the laboratory by hand or under natural conditions by insects, from a (+) mycelium to a (-) mycelium, where they might germinate and fuse with the (-) mycelium, thus converting it into a diploid mycelium; and, conversely, that (-) oidia might be carried from a (-) mycelium to a (+) mycelium, where they might germinate and fuse with the (+) mycelium, and thus convert it into a diploid mycelium.

Since oidia are produced on the haploid mycelia of so many Hymenomycetes, it is obviously of considerable importance to elucidate by experimental means exactly what the function of the oidia is. The problem of the function of the oidia was suggested by Professor Buller to the writer, and its solution is offered in the following pages.

II. MATERIAL AND METHODS.

The fungus chosen as material for this investigation was *C. lagopus*. This well-known species is readily obtained in horse-dung cultures, and its life-history has been worked out by Brefeld (2), Bensaude (1), Buller (4, 5, 6), Hanna (12, 13), Mounce (14), Newton (15), Oort (16), and others.

Spores of *C. lagopus* were obtained in the following manner. Fresh horse-dung was procured from a stable in Winnipeg, and was placed in

a large crystallizing dish covered with a glass plate. The dish was set upon the laboratory table where it was exposed to light. In about ten days fruit-bodies began to appear upon the culture, and these were identified by Professor Buller as *C. lagopus*.¹ Spores from one of the pilei were allowed to fall on a sterilized glass slide. From this spore-deposit single spores were removed by the dry-needle method described by Hanna (11), and they were sown in hanging-drops of cleared dung-agar. In this way a series of monosporous mycelia was obtained.

The medium used for cultivating the fungus was dung-agar, which was prepared as follows: a litre of water was added to 200 grams of fresh horse-dung, and this was boiled in an enamel dish for fifteen minutes. The decoction was then filtered once through cheese-cloth and once through cotton-wool, after which 12 grams of agar were added to the filtrate. The mixture was heated in an Arnold sterilizer for one hour to melt the agar, then tubed, and finally sterilized in an autoclave for one hour at 15 pounds pressure.

To clarify the medium, and thus make it of more use for observation of the mycelium under the microscope, egg-white was employed. The whites of four eggs, after being added to 50 c.c. of water, were slightly beaten and then poured into the dung-agar decoction. This mixture was heated for one hour in flowing steam, filtered through cotton-wool, and tubed.

Malt-agar was occasionally used as a clear medium. It was prepared by boiling 25 grams of ground malt in one litre of water and then adding 12 grams of agar, after which it was filtered and tubed as in the preparation of dung-agar.

The mycelium of *C. lagopus* grows as well upon malt-agar as upon dung-agar.

Each monosporous mycelium, after growing for a few days in a hanging-drop of dung-agar, was transferred to a Petri dish containing a layer of sterile dung-agar about 2 mm. thick.

To keep mycelia, stock-cultures were made by transferring the mycelia to test-tubes 3 in. long and 0.9 in. in diameter, which had been filled about one-third with fresh horse-dung, plugged with cotton-wool, and sterilized in steam at 15 lb. pressure for one hour.

Ten mycelia, nos. 1-10 inclusive, were paired on dung-agar in all possible ways; and, a few days later, each pair was examined to find out whether or not clamp-connexions had appeared upon the hyphae. On the basis of this criterion (12), it was then possible to sort out the ten mycelia into the four well-known sexual groups (*AB*), (*ab*), (*Ab*), and (*aB*).

¹ For illustrations of the fruit-body of *C. lagopus*, vide A. H. R. Buller, *Researches on Fungi*, vol. iii, Figs. 130-8, pp. 300-16; also A. H. R. Buller and D. E. Newton, *Ann. Bot.*, vol. xli, 1927, Pl. XXVIII.

In the experimental work described in the following pages, the mycelia most employed were those numbered 5 and 10. These were sexually opposite. To mycelium No. 5 was given the symbol (*ab*) and to mycelium No. 10 the symbol (*AB*).

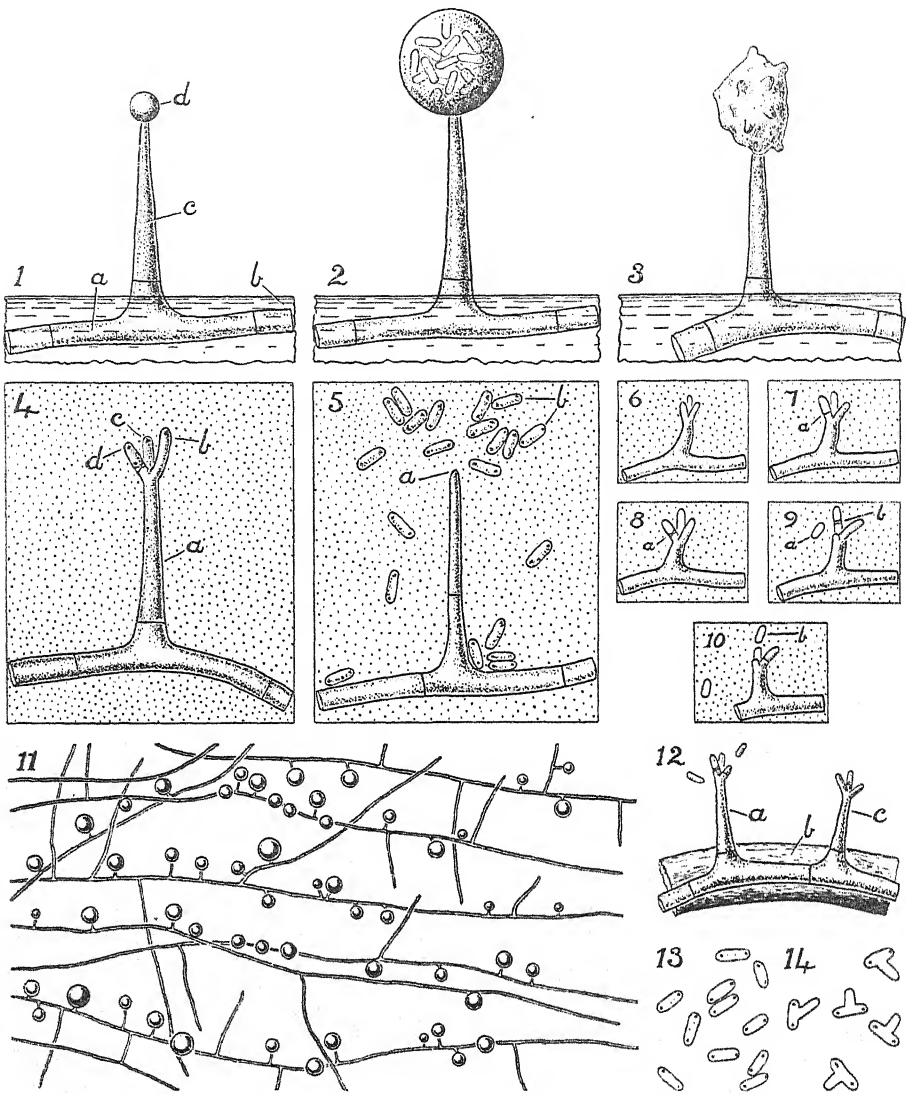
III. THE STRUCTURE AND DEVELOPMENT OF THE OIDIAL FRUCTIFICATIONS.

From two to three days after the germination of a single spore of *C. lagopus* in a culture medium, the haploid mycelium which is developed begins to produce oidia on special lateral branches called *oidiophores*.

On dung-agar plates not covered with a film of water or on horse-dung, the oidiophores grow more or less perpendicularly outwards from the substratum into the air and there produce their oidia in terminal masses (Text-figs. 1-3 and 11). On the other hand, when—as in a hanging-drop of dung-agar in a van Tieghem cell—the culture medium is covered with a film or thin layer of water, the oidiophores are not able to push out from the medium into the air but, instead, they develop and produce oidia in the film of water (Text-figs. 6-10). When oidiophores develop in a film of water, the mycelium, the oidiophores, and the oidia all lie in one plane, and the stages in the development of the oidiophores can be readily followed with the microscope.

An oidiophore which develops in a film of water in the manner described above arises as a hyphal outgrowth of a short cell of the haploid mycelium and attains an average length of 0.05 mm., varying up to 0.1 mm. (cf. Text-figs. 4-5 and 6-10). At its basal end, a well-developed oidiophore (Text-figs. 1 and 2) is 5-6 μ thick, or as thick as the hypha to which it is attached, but it tapers upwards so that its apical end is only 2.5-3.0 μ thick. A little way above its base it is divided by a septum into two cells. Occasionally the oidiophore is divided by two septa into three cells. The protoplasm in the cells of the oidiophore is distinctly vacuolate and exhibits numerous shining particles.

The pointed end of a full-grown oidiophore gives rise to short branches which may be called *oidial hyphae* (Text-fig. 4, *b*, *c*, *d*) and these soon break up into chains of from two to five oidia. The process of oidial formation on an oidiophore which was submerged in a film of water is illustrated in Text-figs. 6-10. At 10.30 a.m. an oidiophore with its oidial branches (Text-fig. 6) was examined and sketched. After twenty minutes (10.50 a.m.) a fine line appeared about one-third of the way from the base of one of the oidial hyphae (Text-fig. 7, *a*) and, after forty-five minutes, a clear space or gap in the protoplasm appeared where the line had been (Text-fig. 8, *a*). Thus an apically-formed oidium became marked off from the basal portion of the oidial hypha. The newly-formed oidium then



TEXT-FIGS. 1-14. *Coprinus lagopus*. 1. A young oidal fructification produced aerally on a four-days-old haploid basidiosporous mycelium growing on dung-agar: *a*, a hypha of the basidiosporous mycelium; *b*, the dung-agar medium in which it is growing; *c*, the oidiophore; *d*, a drop of liquid just beginning to be excreted. $\times 933$. 2. The same oidal fructification ten hours later. The drop has increased greatly in size, and oidia which have been formed within the drop are seen floating about in it. $\times 933$. 3. An oidal fructification which has been exposed to dry air so that the liquid surrounding the oidia has dried up leaving their outlines projecting from the mass. $\times 933$. 4. A young oidiophore on a ten-days-old haploid mycelium. The oidiophore has been immersed in the film of water covering the surface of a dung-agar plate: *a*, the oidiophore; *b*, *c*, and *d*, oidal branches which break up into from two to five oidia. $\times 933$. 5. An old oidiophore on a ten-days-old haploid mycelium. The oidiophore has been immersed in the film of water covering the surface of the agar, and the oidia which were formerly in a mass on the apex of the oidiophore have been scattered: *a*, the rounded tip of the mature oidiophore; *b*, the oidia. $\times 933$. 6-10. Successive stages in the process of oidia formation on an oidiophore developed in the film of water covering the agar. 11. A network of mycelial hyphae with numerous small, round oidia attached to them. 12. A young oidiophore on a ten-days-old haploid mycelium. 13. A group of scattered oidia. 14. A group of scattered oidia.

began to sway from one side to the other about its point of attachment to the hypha. This swaying, apparently due to Brownian movement, continued until the oidium broke loose, which it did forty minutes after the gap was first observed. A gap then appeared on another branch (Text-fig. 9, *b*) and, after thirty minutes, the oidium cut off above the gap began to sway and was then soon set free (Text-fig. 10, *b*).

The oidiophore just described grew in a film of water, i.e. somewhat abnormally; it was shorter than usual and its oidial branches produced only one oidium at a time. Normal oidiophores which project into the air (Text-figs. 1-3 and 11) are much longer and their oidial branches break up into chains of from two to five oidia. In the oidial fructifications shown in Text-figs. 1-3 and 11, the oidial branches are obscured by the drop of liquid in which they are immersed.

The formation of a chain of two or three oidia from an oidial branch is due to: (1) the division of the protoplasm into two or three separate masses; (2) the shrinking or condensation of these protoplasmic masses with the formation of short water-filled gaps or spaces between them and at the base of the oidial hypha; (3) the development of a wall at each end of each shrunken mass of protoplasm; and (4) the dissolution of the wall of the oidial hypha around the water-filled spaces. The two or three oidia set free from an oidial hypha are so small that, if liberated into water, they soon separate from one another and move away (Brownian movement) from their place of origin.

After an oidiophore has ceased to produce oidia and its oidia have been dispersed by immersing the oidiophore in water, the apex of the oidiophore can be seen to be rounded off and not showing any trace of the oidial branches to which it gave rise (Text-fig. 5).

A striking phenomenon in connexion with the production of oidia on the end of an oidiophore which is projecting from horse-dung or from dung-agar into the air is the *excretion of a drop of liquid* at the apex of the oidiophore in such a way that the oidia are immersed in it. Neither Brefeld nor Falck, both of whom studied the oidia of *C. lagopus* and other Coprini, appear to have noticed the drops in question; but, as we shall see, these drops are of great importance for the dispersal of the oidia and their conveyance to a place where they may function to the advantage of the

surface of a hanging-drop of dung-agar. In Fig. 7 a line has appeared at *a*; in Fig. 8 the line has widened into a gap, *a*; in Fig. 9 the oidium *a* has floated freely away from the oidial branch on which it was formed, and another gap is appearing at *b*; in Fig. 10 the oidium *b* has also been freed. $\times 400$. 11. A forty-eight-hours-old haploid mycelium growing on the surface of a poured-plate of dung-agar showing the hyphae with their wide angle of branching and the oidial fructifications. $\times 87$. 12. Oidial fructifications found under natural conditions on horse-dung, sketched from material in water on a glass slide: *a* and *c* the oidiophores; *b*, a bit of straw from the horse-dung. $\times 466$. 13 and 14. Oidia of *Coprinus lagopus* sketched in water on a slide: Fig. 13, some normal oidia showing a refractive granule at each end of each oidium: Fig. 14, a few branched oidia which are occasionally produced. $\times 600$.

species to which they belong. The development of the drops on the tips of the oidiophores was observed in hanging-drops of dung-agar and will now be described.

When an oidiophore first pushes upwards from its substratum into the air, it is a simple tapering hypha with one cross-wall near its base. An hour after it has attained its full length there appears on its tip a tiny liquid drop (Text-fig. 1). The drop increases in size until, at the end of ten hours, it has become about 0.08 mm. in diameter (Text-fig. 2). From the apex of the oidiophore oidial branches are sent out into the drop, where they break up into oidia (cf. Text-fig. 4). In the course of several hours, numerous oidia are thus formed, and they can be seen with the microscope enveloped in the fluid. Apparently the growth of the drop is proportional to the number of oidia that are produced within it. Under normal conditions, the drop is always spherical, and it completely envelops all the oidia contained within it.

Some oidial fructifications which pointed vertically upwards were examined laterally through a horizontal microscope with a magnification of about 400. It was then seen that the oidia practically filled the interior of each drop.

When oidia have been produced in drops of liquid on the apices of oidiophores developed on a dung-agar plate (Text-fig. 11), and one removes the cover and examines the drops with the high power of the microscope, one can often observe that the oidia in the drops exhibit a more or less violent movement. In some drops the movement is typically Brownian; but in others the whole mass of oidia, in the course of a few seconds, may whirl round and round and thus display an activity which to the writer was at first very unexpected. It was found: (1) that whirling can be started by breathing upon the drops, and that the whirling ceases as soon as the drops are no longer breathed upon; and (2) that one can cause a drop to whirl in a clockwise or a counter-clockwise direction by breathing more on the left or on the right of the drop respectively. Thus whirling can be caused by friction with air-currents.

When one looks down on the top of an oidial drop with the high power of the microscope, one sees an enlarged image of some of the oidia immersed in the drop. This is due to the drop acting like a magnifying glass.

Since the drops on the oidiophores are only about 0.05 mm. in diameter, it has not been possible to analyse them chemically; but some simple observations seem to show that the fluid of which they are composed is not pure water but contains colloid matter. When a cover-glass is lowered gently so that it just touches the liquid drops of numerous oidiophores projecting above the surface of dung-agar on which the parent mycelium is growing, and is then lifted up and examined with the microscope, one

observes that the drops with the oidia have come away intact, have dried up, and are attached to the cover-glass as flat circular masses of oidia and dried-up liquid (Text-fig. 20). It was found that, if the cover-glass was dipped in water before the oidiophore liquid had dried, the liquid dissolved in the water and the oidia were dispersed. Two days after some drops were collected and allowed to dry on a cover-glass, it was found that they could not be dissolved either in water or in alcohol.

The oidia in individual drops obtained on a cover-glass were counted. Large drops contain eighty or more oidia, and very small drops five or ten oidia. Drops of average size with a diameter of 0.05 mm. contain about twenty oidia.

Very large oidial drops may be formed by the fusion of several smaller drops and by further excretion of liquid. A continuation of this process may result in almost the entire surface of a haploid mycelium growing on a plate of dung-agar becoming covered with closely-packed oidia.

A photograph showing the appearance of the oidial fructifications when seen with a magnification of 100 is reproduced in Pl. X, Fig. 2. When the drop on an oidiophore is allowed to dry it shrivels and assumes a rough appearance owing to the fact that the ends of the oidia project (Text-fig. 3).

The individual oidia of *C. lagopus* are $1\ \mu$ in width and may be from $2\ \mu$ to $10\ \mu$ long. The average length is about $5\ \mu$. Occasionally an oidium is produced which has a side branch, as shown in Text-fig. 14. Two refractive granules are usually to be seen in the protoplasm within each oidium (Text-figs. 5, 13, 14).

Bensaude (1) stained the oidia of *C. fimetarius*, and she illustrated the stained oidia in her paper on Pl. II, Figs. 3 and 4, and on Pl. IV-V, Fig. 4. In these illustrations, one nucleus can be seen in each oidium, and some of the nuclei can be seen undergoing division.

IV. THE CONDITIONS UNDER WHICH OIDIAL FRUCTIFICATIONS ARE PRODUCED.

The oidia of *C. lagopus* are produced by haploid mycelia only, never by diploid. It is true that a haploid mycelium which has been converted into a diploid mycelium may still have attached to it oidia which were produced when the mycelium was haploid; but, with the advent of the diploid phase, the mycelium ceases to produce oidia. The same is true for *C. niveus* and *C. curtus*—two other heterothallic species of *Coprinus* which have been examined by the writer.

Brefeld (2) showed that *C. stercorearius*, now known to be a homothallic species, never produces oidia; and Falck (9) demonstrated that *C. sterquilinus*, also now known to be homothallic, is entirely without oidia.

The writer kept monosporous mycelia of *C. stercorearius* under observa-

tion from the time of germination of the spores onwards for several days. Three days after the germination of each spore the mycelium which developed from it became diploid. No oidia whatever were produced.

It is a rather remarkable fact that a *very young* haploid mycelium of *C. lagopus* produces oidia for a time, even when it is growing in contact with mycelia of opposite sex. A large number of spores (two hundred or more), and therefore spores of all the four sexual types (*AB*), (*ab*), (*Ab*), and (*aB*), were sown together in a hanging-drop of cleared dung-agar. Twelve hours later about one-fourth of the spores had germinated. When two to five days old, the young mycelia were vigorously producing oidia, each mycelium behaving as though it were isolated from the rest of the mycelia. Fusion between the mycelia of opposite sex then began to take place, and, in the course of twenty-four hours, all of the leading hyphae in the hanging-drop developed clamp-connexions and ceased to produce oidia. It may be concluded that a young spore-mycelium passes through a period of oidia-production of about forty-eight hours' duration, and that it will not unite with a mycelium of opposite sex until this period has been completed.

The number of oidia produced by a haploid mycelium developed from a spore on one square millimetre of dung-agar varies for different mycelia and for different parts of the same mycelium. Some mycelia produce far more oidia than others, but all haploid mycelia produce them in great numbers. In what seemed to be an average mycelium, a count showed that there were fifteen to twenty oidial fructifications per square millimetre of dung-agar surface. At this rate, on a mycelium covering a square with each side 5 cm. (2 inches) long, the number of oidial fructifications would be 2,500. Reckoning twenty oidia to each oidial drop, such a square of mycelium would give rise to 50,000 oidia. It is possible that, after the drops become fused together in the older part of the mycelium, in the almost continuous fluid layer so produced still more oidia are developed, thus greatly increasing the calculated number. Some idea of the great number of oidia which a haploid mycelium of *C. lagopus* may produce may be gained by a glance at the photomicrograph reproduced in Pl. X, Fig. 2.

The oidial fructifications on haploid mycelia derived from spores normally develop in the air, and never under the surface of the medium. It therefore appears that air is necessary for their development.

If a sterilized dung ball is inoculated with a haploid mycelium, the mycelium grows very vigorously and becomes fluffy owing to the production of aerial hyphae. The oidial fructifications are produced in the same way as on dung-agar: the surface of the dung ball becomes covered with them, and the oidiophores project away from the substratum.

The cover was removed from an agar plate in which a haploid mycelium was growing, and the plate was allowed to rest in an inverted position on two corks on the laboratory table. The dish and corks were covered with

a bell-jar under the edge of which a small slice of cork was placed to allow free access of air. Thus the surface of the agar was exposed to air much drier than that in a closed Petri dish, and yet not dry enough to dry the agar surface completely. At the end of three days the oidiophores were being produced by the leading hyphae in as great numbers as under the conditions of excess of moisture existing in a closed Petri dish. Under the dry conditions, however, there were on the oidiophores not as many large compound drops due to fusion of individual drops as may be observed under more moist conditions.

Light is not essential for the production of oidia, for it was found that oidia were produced in cultures kept for ten days in the dark. Occasionally, the mycelium on agar plates shows concentric rings, the oidial fructifications being produced to a greater and lesser extent alternately. Some experiments were undertaken to determine whether or not the ring-formation is due to the alternating influence of light and darkness. Mycelia on agar plates were exposed to sunlight for one hour each day for ten days, and were kept in the dark for the rest of the time. This treatment failed to produce the concentric rings. Further investigation is desirable to explain the phenomenon of the concentric ring growth-habit.

V. THE OCCURRENCE OF OIDIA IN NATURE.

Although oidial fructifications are produced on dung-agar, &c., the laboratory conditions are somewhat artificial, and it seemed desirable to find out whether or not the oidial fructifications are produced under natural conditions.

A large number of fruit-bodies of *C. lagopus* were noticed coming up on unsterilized horse-dung which had been brought into the laboratory and placed in a covered glass vessel sixteen days previously. This culture was examined in the hope of finding oidia. Under the low power of the microscope many oidial fructifications were seen projecting above the dung surface, and there were many more on strands of aerial mycelium. These oidial fructifications resembled in appearance those which had been studied in laboratory cultures of haploid mycelia of *C. lagopus*. Some oidial fructifications were found on a bit of straw, and the straw was then removed from the rest of the dung with fine forceps and placed on a glass slide in a drop of water. Hyphae with simple cross-walls, from which oidiophores bearing oidia projected, were then seen (Text-fig. 12).

On three other occasions oidial fructifications have been found in unsterilized stable-dung cultures. The dung was obtained fresh from a stable and oidial fructifications exactly resembling those of *C. lagopus* were found upon it two to three days after it had been deposited. About a week later, on the same dung, appeared numerous fruit-bodies of *C. lagopus* as well as some fruit-bodies of *C. curtus*, &c. From these observations it

is clear that oidial fructifications are produced under natural conditions in the open even in competition with other fungi, bacteria, &c. Since, in the cultures under discussion, *C. lagopus* fruit-bodies were more numerous than those of any other fungus, and since the structure of the oidiophores and oidia exactly resembled that of the oidiophores and oidia produced in pure cultures of *C. lagopus*, there can be little doubt that the oidiophores and oidia found in the wild cultures were produced by *C. lagopus*. So far as the writer is aware, this is the first time that oidiophores and oidia of any species of Hymenomycetes have been observed under natural conditions.

VI. THE GERMINATION OF THE OIDIA.

Some spores of *C. lagopus* were sown separately in hanging-drops of cleared dung-agar and malt-agar. The spores germinated, and each of the monosporous mycelia soon began to produce numerous oidiophores and oidia. Owing to the fusion of oidial drops, and to the drops coming into contact with the dung-agar, great numbers of the oidia became scattered over the surface of the medium. When these cultures were ten days or more old oidia were observed germinating in them *in situ*.

An oidium when about to germinate becomes much swollen (Text-fig. 17, *b, c*), rather more at the ends than in the middle, and the refractive granules at the ends are then more prominent than in ungerminated oidia (Text-fig. 17, *b*). The swollen oidium usually develops a single germ-tube at one end (Text-fig. 17, *d*), but sometimes it sends out two germ-tubes, one from each end (Text-fig. 17, *f*). The width of the germ-tube is about two-thirds that of the swollen oidium; and, as the germ-tube is produced, large vacuoles appear in the oidium, thus indicating that the germ-tube is being produced at the expense of the protoplasm of the oidium (Text-fig. 17, *d*). Germ-tubes which (as in ten-days-old cultures) are produced on an exhausted medium develop slowly, often soon cease to elongate, and not infrequently anastomose with one another (Text-fig. 17, *e*).

An attempt was made to germinate the oidia in a freshly prepared nutrient medium. Oidia of various ages were sown in hanging-drops of one or other of the following: water, sugar solution, cleared and uncleared dung-agar, and 2.5 per cent. malt-agar. The results in these first experiments were all negative, this notwithstanding that similar oidia to those employed had been seen germinating *in situ*.

The effect of heat on the germination of oidia was next investigated. Oidia were obtained on each of a number of cover-glasses by bringing the cover-glasses lightly into contact with the surface of a thirty-days-old culture of a haploid mycelium. The cover-glasses with the oidia attached to their lower surfaces were then placed on van Tieghem cells which had been partly filled with water. The preparations were then heated from above

by means of a desk-lamp to 50° C., were kept at this temperature for one minute, and were then allowed to cool. Some control preparations were not heated.

In the course of twenty-four hours the oidia which had been heated had swollen and germinated, while the oidia in the unheated control preparations had not germinated. This experiment was repeated several times, always with the same result. It thus appears that heating in some way assists oidia to germinate.

Next, some oidia taken from a three-weeks-old culture of the haploid mycelium No. 5 were set in hanging-drops of cleared dung-agar and malt-agar. They germinated rapidly and in large numbers. After this, oidia of various ages (quite young to several weeks old) were taken from the haploid mycelia Nos. 5 (*ab*), 10 (*AB*), 2 (*Ab*), and 7 (*aB*) and were sown in hanging-drops and poured-plates containing the same media; again germination took place readily. Not a single one of upwards of twenty of these cultures yielded a negative result, for germinating oidia were observed in every one of them.

Five months after the experiments recorded above were performed, oidia were taken from the haploid mycelia Nos. 3 (*AB*), 6 (*ab*), 4 (*Ab*), and 1 (*aB*) and were sown in hanging-drops of freshly prepared dung-agar. Again, in every drop, the oidia germinated freely.

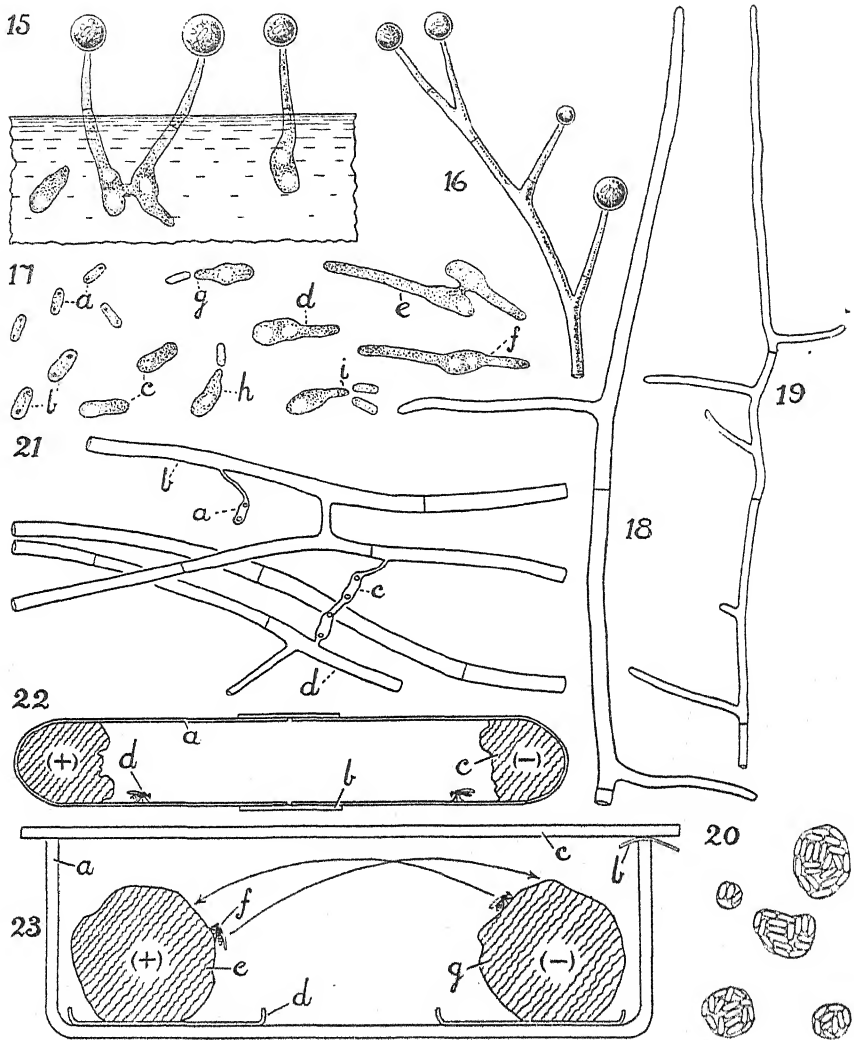
The two sets of experiments just described showed that the oidia of eight of the ten monosporous mycelia isolated for use in the present investigation germinated readily when subjected to favourable conditions.

One may ask: why is it that in the first set of experiments in which the culture medium consisted of hanging-drops of dung-agar and malt-agar the oidia failed to germinate, whereas in similar experiments made subsequently the oidia germinated well? Possibly the answer is to be sought in the variability of the horse-dung used in making the media. It is unlikely that the media made at different times with horse-dung are ever quite alike in composition. Even after it is made, dung-agar undergoes change, for its colour gradually becomes darker.

There can be little doubt that, when subjected to suitable conditions, the oidia of *C. lagopus* germinate readily. This conclusion is supported not only by the germination experiments already described, but by other experiments made with the help of flies, to be described in a later section.

When oidia were sown in hanging-drops of dung-agar or malt-agar the minimum time for germination after sowing was found to be eight hours, and it was estimated that in each of the cultures about 80 per cent. of the oidia germinated.

The germ-tubes of oidia show a lesser tendency to anastomose in a fresh culture medium than in an exhausted one. In a fresh medium some of the germ-tubes do not grow much longer than the oidia which



TEXT-FIGS. 15-23. *Coprinus lagopus*. 15. Oidia of one sex which have germinated in dung-agar and have given rise to short germ-tubes. The germ-tubes have projected into the air to become oidiphores, and each of them bears at its apex the usual mass of oidia and liquid excretion. $\times 933$. 16. A branched oidiphore produced on a mycelium derived from oidia of one sex, growing on dung-agar or horse-dung. $\times 400$. 17. Oidia sown in a hanging-drop of cleared dung-agar; *a*, ungerminated oidia; *b* and *c*, much swollen oidia about to germinate, eight hours after having been sown; *d*, a germinating oidium showing two vacuoles within and producing a germ-tube from one end only; *e*, two germinating oidia which have anastomosed; *f*, a germinating oidium which has produced a germ-tube from each of its ends; *g* and *h*, oidia each with a single germ-tube from the end of which has been constricted off a second-generation oidium; *i*, a germinating oidium with a germ-tube which has formed two new oidia. $\times 933$. 18. A leading hypha of a haploid mycelium derived from a basidiospore, developed on dung-agar. The hypha is 4μ to 5μ in diameter. $\times 400$. 19. A leading hypha of a mycelium derived from an oidium, developed on dung-agar. The hypha is 2μ to 3μ in diameter. $\times 400$. 20. Masses of oidia which have become attached to a cover-glass by allowing the cover-glass to come lightly into contact with the surface of a haploid mycelium growing on a dung-agar plate. Each mass of oidia is held together by dried-up oidiphore fluid. $\times 400$. 21. To show the fusion of the oidal germ-tubes of the

produced them before they begin to form new oidia, as shown in Text-fig. 17 *g, h, i*. Often these germ-tubes grow out of the medium into the air, and there soon develop into oidiophores bearing the usual drop of fluid containing oidia (Text-fig. 15). The oidiophores thus produced are usually smaller in size than those produced on ordinary haploid mycelia developed directly from basidiospores. While some oidia develop in the manner just described many others in the same cultures give rise to an extensive mycelium which grows vigorously, spreads indefinitely through the medium, and produces great numbers of aerial oidiophores bearing the usual ooidal drops.

The oidia which are developed on a mycelium derived from a basidiospore may be considered as *first-generation* oidia. If one sows these oidia, the mycelium which they produce soon develops a fresh crop of oidia—*second-generation* oidia. If one sows second-generation oidia one can obtain *third-generation* oidia; and so forth. Twelve successive generations of oidia were thus obtained, and the twelfth-generation oidia did not differ appreciably in size and power of germination from those of the first generation.

The technique for sowing the twelve successive generations of oidia was as follows. A drop of cleared dung-agar was placed on the under-side of a sterilized cover-glass and then the drop was brought lightly into contact with the surface of a haploid mycelium growing on dung-agar in a Petri dish. The cover-glass was then set on the glass ring of a van Tieghem cell which contained a little water. The germination of the oidia required about eight hours, and in the next four hours the germ-tubes and mycelia produced new oidia in abundance. From one to a few days elapsed between the sowings of any two successive generations of oidia.

The mycelium derived from a basidiospore, No. 5 (*ab*), which produced the first of the twelve successive generations of oidia, developed clamp-

oidia *a* and *c* of one sex (+) with the hyphae *b* and *d* of a mycelium derived from a basidiospore of opposite sex (-). 22. Diagram of an apparatus used for investigating the relation of flies with the oidia of *Coprinus lagopus*. Two wide test-tubes *a*, each containing a mass of sterilized horse-dung *c* have been fitted mouth to mouth, and have been bound together by means of the cardboard collar *b*. On one dung-mass is a haploid mycelium derived from a basidiospore of one sex (+), and on the other dung-mass is another haploid mycelium derived from a basidiospore of opposite sex (-). The (+) mycelium has developed (+) oidia at its surface, and the (-) mycelium (-) oidia. Some *Drosophila* flies *d* have been introduced into the chamber. They carry (+) oidia from the (+) mycelium to the (-) mycelium and (-) oidia from the (-) mycelium to the (+) mycelium, with the result that both the (+) mycelium and the (-) mycelium become diploidized. One-third the actual size. 23. Diagram of another apparatus used for investigating the relation of flies with the oidia of *Coprinus lagopus*; *a*, a large glass crystallizing dish with a glass cover *c* under which a slip of paper *b* has been inserted to make a slight space between cover and dish with a view to ventilation; *d*, one section of a small Petri dish; *e*, a horse-dung ball on which is growing a mycelium derived from a basidiospore of one sex (+), and *g*, another horse-dung ball on which is growing a mycelium derived from a basidiospore of opposite sex (-). The (+) mycelium bears (+) oidia and the (-) mycelium (-) oidia. Some *Drosophila* flies *f* have been introduced into the chamber. They carry (+) oidia from the (+) mycelium to the (-) mycelium and (-) oidia from the (-) mycelium to the (+) mycelium, with the result that both the (+) mycelium and the (-) mycelium become diploidized. One-third the actual size.

connexions when paired with another mycelium derived from a basidiospore, No. 10 (AB). The mycelium derived from the first-generation oidia and the mycelium derived from the second-generation oidia also developed clamp-connexions when paired with mycelium No. 10 (AB). Hence the two mycelia derived from the oidia had the same sexual constitution (*ab*) as the mycelium No. 5 from which they were primarily derived. From these observations we may conclude that successive generations of oidia are all of one and the same sex.

VII. THE MYCELIUM PRODUCED BY OIDIA.

For each of the haplonts Nos. 5 (*ab*) and 10 (AB), the mycelium derived from a *basidiospore* and the mycelium derived from *oidia* have been carefully compared with a view to finding out what differences, if any, there are between them.

As a result of the comparison it has been found : (1) that, whereas the individual hyphae of a basidiosporous mycelium are about $5\ \mu$ thick, those of an oidial mycelium are only about $2\ \mu$ thick, and are therefore relatively *very fine* (cf. Text-figs. 18 and 19); (2) that, under the same culture conditions, an oidial mycelium *grows more slowly* over the culture medium than a basidiosporous mycelium; (3) that, whereas a basidiosporous mycelium produces many aerial hyphae—on this account becoming fluffy—an oidial mycelium, except for its oidiophores, *produces no aerial hyphae* whatever; (4) that, whereas a basidiosporous mycelium produces fruit-bodies (haploid and usually imperfectly developed), an oidial mycelium *remains sterile indefinitely*; (5) that an oidial mycelium produces *far more oidiophores* per unit area than does a basidiosporous mycelium; (6) that, whereas a basidiosporous mycelium produces its oidiophores always aerially or in a film of moisture on the surface of the medium, an oidial mycelium produces oidiophores not only aerially or in a film of water on the surface of the medium, but sometimes also *beneath the surface of the medium*; and (7) that, whereas the oidiophores of a basidiosporous mycelium (at least when first formed) are never freely branched, those of an oidial mycelium are *frequently branched* (cf. Text-figs. 1-5 and 16).

Evidence of (4) the sterility of an oidial mycelium so far as fruit-bodies are concerned was obtained as follows. Oidia taken from the haploid mycelium No. 5 were sown on sterile dung balls in crystallizing dishes and in wide test-tubes (3×1 inch). At the end of twenty-four hours the oidia had grown into a fine white oidial mycelium, and this eventually covered the whole surface of the substratum and produced many thousands of oidiophores. These cultures were kept under observation for three months, yet, during this long period, they showed not the least sign of producing fruit-bodies. On the other hand, a mycelium derived from a basidiospore,

after being set on a sterile dung ball, usually fruits within ten to fourteen days.

In comparative cultures, a glance is sufficient to convince one of the fact that oidial mycelium produces far more oidiophores per unit area of the culture medium than does a basidiosporous mycelium. It was observed that, whereas a basidiosporous mycelium rarely produces more than fifteen oidial fructifications per square millimetre, an oidial mycelium may produce upwards of sixty-five oidial fructifications per square millimetre.

Oidia of opposite sex were sown together in the following manner. A drop of cleared dung-agar was suspended on the under-side of a cover-glass and the drop was allowed to come lightly into contact first with the surface of an agar plate on which the haploid mycelium No. 5 (*ab*) was growing and then with the surface of an agar plate on which the haploid mycelium No. 10 (*AB*) was growing. The cover-glass with the agar on the under-side was then placed on the glass ring of a van Tieghem cell containing a little water. The oidia germinated in the usual manner, and a network of mycelium was produced in which there were many fusions between the individual hyphae. The mycelium which developed after the fusion of germ-tubes of oidia of opposite sex was relatively fine like mycelium derived from oidia of one sex, and it did not develop any clamp-connexions. Many of the hyphae of the network bore the usual oidiophores and oidia.

Oidia taken from the haploid mycelia Nos. 6 and 2 (sexually opposite) were also sown together, with the same result as before: no clamp-connexions appeared on the mycelium developed from the oidia.

It has not been possible up to the time of writing to repeat the above experiments or to investigate the matter further. However, the results of the experiments so far made seem to indicate that, for *C. lagopus*, haploid mycelia of opposite sex derived from oidia of opposite sex do not unite with one another to form a diploid mycelium, and that a diploid mycelium arises through the agency of oidia only when oidia of one sex come into contact with a mycelium of opposite sex derived from a basidiospore.

VIII. THE EFFECT OF SOWING OIDIA ON A HAPLOID MYCELIUM OF OPPOSITE SEX.

When (+) oidia (oidia of one sex) are deposited on a (−) mycelium (a mycelium of opposite sex) they germinate, and the germ-tubes fuse with the (−) mycelium (Text-fig. 21), and the (−) mycelium is converted into a diploid mycelium. Conversely, when (−) oidia are deposited on a (+) mycelium they germinate, and the germ-tubes fuse with the (+) mycelium, and the (+) mycelium is thereby converted into a diploid mycelium. The evidence which led to these conclusions will now be adduced.

A fragment of the haploid mycelium No. 10 (*AB*) was transferred to

malt-agar in a Petri dish where it was allowed to grow until it was about 4 cm. in diameter. A platinum loop was then touched lightly to the surface of a culture of the haploid mycelium No. 5 (*ab*), and oidia from this mycelium adhered to it. The loop with the oidia on it was then touched to the surface of the agar in the plate containing the mycelium No. 10 (*AB*) at a point just in front of the leading hyphae.

Three days after the (*ab*) oidia were added to the plate at the periphery of the (*AB*) mycelium all the leading hyphae of the (*AB*) mycelium had become diploid, as was indicated by the presence of clamp-connexions on them.

The experiment just recorded was successfully repeated four times, i. e. in each experiment mycelium No. 10 was diploidized¹ through the agency of oidia derived from mycelium No. 5.

In another experiment oidia from mycelium No. 10 (*AB*) were sown at the periphery of mycelium No. 5 (*ab*); and the haploid mycelium (*ab*) was soon diploidized thereby.

So many oidia were used as inoculum in the two previous sets of experiments that it was found very difficult to observe clearly just where the germ-tubes from oidia fused with the haploid spore-mycelium. To overcome this difficulty, a few oidia were germinated beforehand in hanging-drop cultures and transferred to the mycelium of opposite sex in an agar plate by touching the hanging-drop to the agar in the plate at a point just in front of the leading hyphae, as in the previous experiments. The oidia were watched continuously, and in several places the germ-tubes were seen to *fuse with the spore-mycelium*, as illustrated in Text-fig 21.

In another set of experiments, oidia from an (*AB*) mycelium, which was growing on a dung ball in a crystallizing dish 12 cm. wide, were gathered on a sterile platinum loop by touching the loop lightly to the mycelium. The oidia were then deposited on an (*ab*) mycelium growing on a dung ball in another crystallizing dish; and, conversely, the oidia from an (*ab*) mycelium were deposited on an (*AB*) mycelium. Both the (*AB*) mycelium and the (*ab*) mycelium became diploid within a week. Control haploid mycelia, which were grown on dung balls in crystallizing dishes but were not inoculated with oidia, remained haploid. This experiment was repeated several times, always with the same result.

The foregoing experiments definitely prove that oidia of one sex are capable of causing a haploid mycelium of opposite sex to become diploid.

IX. WIND AND RAIN AND THE DISPERSAL OF OIDIA.

Wind plays such an important part in the dissemination of seeds and spores that the possibility of oidia being dispersed by the wind has been

¹ For the first use of the terms *diploidize* and *diploidization process*, vide A. H. R. Buller (5).

investigated. Oidial fructifications were watched under the microscope while a sharp puff of air was applied to them by blowing through a rubber tube. The oidiophores shook violently, so that some of them were flattened out on the substratum; but neither when the oidial drops were moist nor when they had been allowed to dry were oidia detached by blowing on them.

When an oidial drop is in the moist condition it can be detached from its oidiophore by *violent* shaking. This was demonstrated as follows. The bottom section of a dung-agar Petri dish in which a large haploid mycelium bearing numerous oidiophores was growing was inverted over the bottom section of another Petri dish containing freshly-poured dung-agar. Then the inverted upper Petri-dish section was violently struck a number of times with the hand. A few of the oidial masses were thereby detached and fell on to the nutrient agar in the lower dish, where they germinated and produced new oidial mycelia. No oidia could be shaken off in this manner when the oidial drops were first allowed to dry. The adhesive nature of the liquid in which the oidia are produced causes the oidia to be bound securely to the oidiophore when the liquid dries, so that the oidia cannot be detached.

The experiments just described indicate clearly that the oidia of *C. lagopus* are not normally dispersed by the wind.

Since the drops in which the oidia are developed are soluble in water when they have just been excreted and have not dried up, it is obvious that rain may scatter the oidia locally. However, rain is a phenomenon of uncertain occurrence and must tend to wash the oidia to the ground rather than to transport them from place to place.

X. THE TRANSPORTATION AND DEPOSITION OF OIDIA BY FLIES AND THE ACTION OF THE DEPOSITED OIDIA ON HAPLOID MYCELIA.

The oidial drop on an oidiophore is very mucilaginous and adheres readily to any object brought into contact with it. When a cover-glass is lowered so as to touch the oidial drops on a mycelium they come away from their oidiophores intact. The end of a glass rod, after having been rubbed lightly over the surface of a haploid mycelium, is covered with oidial fluid and oidia. Oidial drops can also be readily removed from their oidiophores by means of a platinum loop.

It seemed probable that, if an insect were to come into contact with some oidial fructifications, the oidia would adhere to its legs and body. The experiments described in the following pages show that insects actually do transport oidia from one place to another.

The species of insect used in these experiments was *Drosophila melanogaster*, the celebrated fly employed by Morgan and his pupils for the study

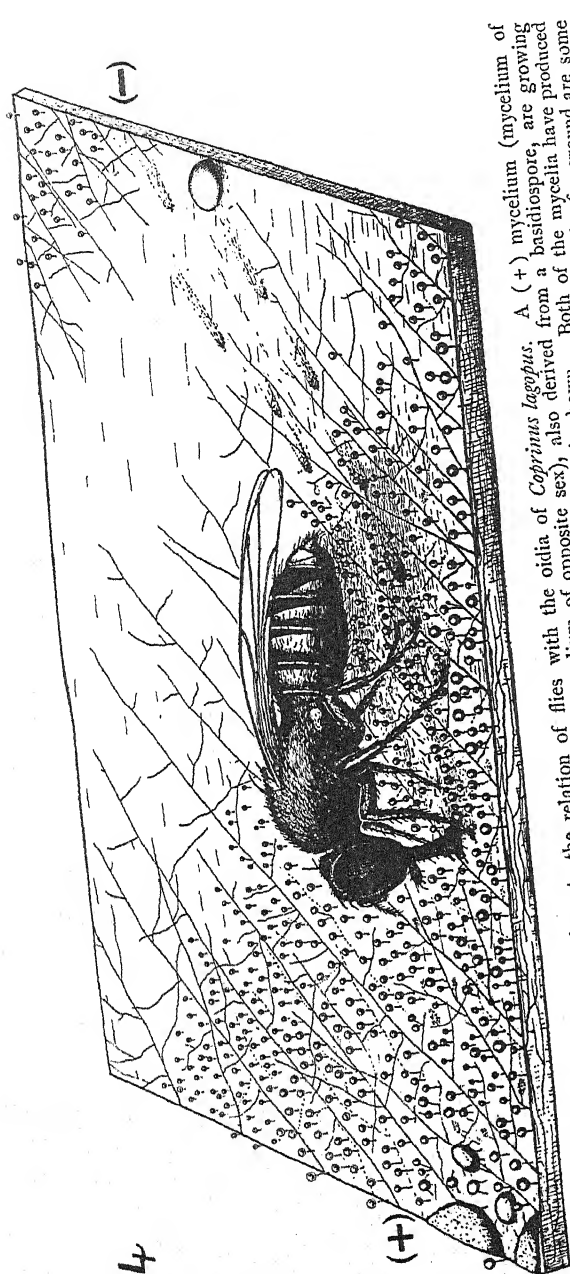
of genetics. The flies were reared in pint sealers (glass jars) plugged with cotton-wool. Over-ripe banana was provided as food.

The bottom section of a Petri dish in which a haploid mycelium (*ab*) was growing on a layer of dung-agar was fitted against the bottom section of another Petri dish containing a layer of sterile dung-agar, and a fly was introduced into the chamber thus formed. The two Petri dish sections were then fastened together by means of a wide strip of adhesive paper pasted around their rims. The fly was allowed to walk over the mycelium contained in one of the plates for half a minute. Then the chamber was suddenly held up vertically and turned by the hand, so that light from a window streamed through the base of the plate containing the sterile agar. Immediately the fly, in response to the heliotropic stimulus, left the plate containing the mycelium for the plate containing the sterile agar, and here it walked about over the surface of the medium. The plates were then disjoined, the fly was removed, and a cover was placed over the plate which, until the advent of the fly, had contained sterile agar.

One day after the fly had walked on the sterile plate the bacteria which had been deposited from the fly's feet on the agar had formed bacterial colonies. These colonies clearly marked out the track of the insect. On the agar surface where the fly had walked large numbers of oidia were observed with the microscope. These germinated and developed mycelia which soon pushed their way far beyond the bacterial colonies and rapidly developed new oidiophores and oidia. Five days after the fly had walked over the plate the plate was photographed, and the photograph, reproduced in Plate X, Fig. 1, clearly shows the fly-tracks with bacterial colonies down the centre and oidial mycelium growing outwards on all sides. Some of this mycelium was removed and paired with some (*AB*) mycelium derived from a basidiospore, with the result that clamp-connexions were formed. It was thus proved that the oidial mycelium had the constitution (*ab*), i.e. the same constitution as the mycelium over which the fly had walked. The fly which had walked over the (*ab*) mycelium was examined under the microscope, and masses of oidia were found clinging to its feet, especially to the tarsal claws and the hairs on the tarsi.

The experiment just described shows conclusively that flies can transport oidia and that the oidia, after transportation, can germinate and produce mycelia.

In another experiment six flies were allowed to go without food for twelve hours. They were then put into a Petri dish in which a haploid mycelium was growing on dung-agar, and the behaviour of the flies was observed with the low power of a binocular microscope. Immediately upon being set free in the dish each hungry fly began to suck up the oidial drops with its proboscis. The lower end of the proboscis of the *Drosophila*



TEXT-FIG. 24. A diagram to illustrate the relation of flies with the oidia of *Coprinus lagopus*. A (+) mycelium (mycelium of one sex), derived from a basidiospore, and a (-) mycelium (mycelium of opposite sex), also derived from a basidiospore, are produced near one another on dung-agar in a Petri dish. Only a small portion of each mycelium is shown. Both of the mycelia have produced numerous aerial oidiophores, each crowned with a drop containing many free-floating oidia. At the extreme left in the foreground are some large masses of oidia. These have been formed by the fusion of oidia drops which have grown and touched one another and the surface of the agar. A fly (*Drosophila melanogaster*) has walked over the (-) mycelium (right background) and has carried (-) oidia on its feet and proboscis to the (+) mycelium. The (-) oidia thus deposited will germinate, their germ-tubes will fuse with the hyphae of the (+) mycelium. The fly has deposited a drop of excrement on the surface of the agar between the two mycelia. This drop contains large numbers of oidia which will germinate and produce a mycelium. The fly is now walking over the (+) mycelium and is engaged in sucking up the oidia drops on the tops of the last segment of its hind legs and will be deposited elsewhere when the fly moves on. The oidia which the fly is now taking into its alimentary canal will be deposited in a viable condition in its excrement. x about 20.

fly is about 0.3 mm. wide, while the oidial fructifications are about 0.1 mm. in length, and the oidial drops about 0.05 mm. in diameter. The end of the fly's proboscis, therefore, touched perhaps a dozen oidial drops at the same time. The flies walked along, vigorously sucking up the drops from the oidiophores and pressing down the oidiophores on to the agar surface, thus leaving a cleared trail through the projecting oidial fructifications (Text-fig. 24). Often a fly stopped feeding to rub its proboscis with its feet and so clean off the adherent liquid.

A clean glass plate was now substituted for the cover of the Petri dish and the Petri dish was inverted to allow the flies to walk on the glass plate. The flies were watched, and as soon as several drops of excrement had been deposited which had not been touched by the flies' bodies or feet the plate was removed (a fly-drop is shown on the right-hand side of Text-fig. 24).

The fly-drops were examined under the microscope and were seen to contain many oidia as well as some yeast-cells which are normally carried in the alimentary canal and on the bodies of the fruit-flies. To some of the fly-drops malt-agar was added as a nutrient medium. Other drops were enclosed in water-containing vanTieghem cells without additional nutriment. Under both sets of conditions the oidia in the fly-drops germinated within twenty-four hours and the mycelium derived from the germ-tubes gave rise to new oidial fructifications.

The foregoing observations justify the conclusion that flies transport oidia in two ways: (1) on their legs and bodies; and (2) by using the oidial liquid as food, sucking up the oidial drops, passing the oidia through their alimentary canals, and depositing the oidia in viable condition in fly-drops.

Other dung-inhabiting flies, beetles, and other insects must also be capable of transporting oidia on their legs and bodies.

Experiments will now be described in which it will be shown that flies can carry oidia from a (+) mycelium to a (−) mycelium or from a (−) mycelium to a (+) mycelium, and that the oidia may function in such a way as to convert haploid mycelia into diploid mycelia.

In the first set of experiments (Text-fig. 23) two balls of fresh horse-dung were placed as far apart as possible (a distance of 12 cm.) in each of four large crystallizing dishes, 23 cm. in diameter and 8 cm. deep, the bottoms of which were lined with moistened filter-paper. The filter-paper was placed in the dishes to give the flies which were to be put in later a rough surface to walk on and so prevent them from being drowned in the water which accumulated on the bottom of the crystallized dishes. Each ball was placed in an uncovered shallow Petri dish 7 cm. in diameter, an arrangement which prevented the mycelium of one dung ball from growing over the surface of the moist filter-paper in the bottom of the crystallizing

dish and so meeting and mating with the mycelium of the other dung ball. Each crystallizing dish was covered with a glass plate, and the dish along with its contents was sterilized in steam at 15 pounds pressure for one hour.

In each dish one dung ball was inoculated with the haploid mycelium No. 5 (*ab*) and the other with the haploid mycelium No. 10 (*AB*). Two of these dishes were used for experiments with flies and were labelled A and B, and two were kept for controls.

Three days after inoculating the dung balls the mycelium which had developed on each ball covered an area of about two square centimetres. Some *Drosophila* flies were then removed from the glass jar in which they had been reared to another jar. Here they were etherized, and then eight of the etherized flies were placed in each of the experimental dishes A and B (cf. Text-fig. 23). No flies were placed in the control dishes.

In the course of the next few days the flies in each dish flew back and forth from the mycelium on one dung ball to the mycelium on the other. They could also be seen sucking up the drops of liquid containing the oidia.

One week after the flies had been put in the dishes, a piece of the mycelium on each dung ball was mounted in water on a glass slide and examined under the microscope for the presence of clamp-connexions. It was found that the control mycelia were still all haploid, whereas *all the mycelia in the experimental dishes were diploid*. This indicates: (1) that the flies had carried oidia from the mycelium No. 5 (*ab*) and had deposited them on mycelium No. 10 (*AB*) and had carried oidia from mycelium No. 10 and had deposited them on mycelium No. 5; (2) that the oidia must have germinated; and (3) that the germ-tubes or hyphae of the mycelium derived from the oidia must have fused with the mycelium on which the oidia had been deposited.

The experiment just described was successfully repeated four times. However, the apparatus employed was found to be defective in several respects. The large crystallizing dishes were cumbersome to handle, and when a small piece of paper was placed under the glass cover at the edge of each dish to allow sufficient ventilation for the mycelium growing in the dish it was found to be difficult to prevent the culture from becoming contaminated with mould.

The experiment was, therefore, modified in the following manner. The haploid mycelia were grown in wide test-tubes containing sterilized horse-dung. The dung in each tube was inoculated with a fragment of one of the following stock mycelia: Nos. 1, 2, 5, 6, 9, and 10. Four days after the six tubes of dung had been inoculated their plugs were removed and they were fitted together mouth to mouth in the manner shown in Text-fig. 22, so as to form three chambers. Each pair of tubes contained two mycelia of

opposite sex, and the pairings were as follows: No. 5 (*ab*) with No. 10 (*AB*), No. 6 (*ab*) with No. 2 (*AB*), and No. 1 (*Ab*) with No. 9 (*aB*). Into each of the three chambers six *Drosophila* flies were introduced, and then the two tubes forming each chamber were bound together by means of a tight cardboard collar 5 cm. wide. The flies were free to wander from the mycelium in one end of each of the chambers to the mycelium at the other end (cf. Text-fig. 22); and they were kept in the chambers for forty-eight hours and then removed. No flies were placed in tubes of each mycelium kept as controls.

The mycelia in the three sets of tubes were examined with the microscope five days after the flies were introduced into the chambers, and all six of them were found to have developed clamp-connexions and therefore to have become diploid. On the other hand, the six mycelia in the control tubes had not developed clamp-connexions and were therefore still haploid.

The experiment was repeated, but this time the flies were allowed to remain in the tubes only fifteen minutes. The results were equally satisfactory: the mycelia visited by the flies became diploid, while the control mycelia remained haploid.

To find out whether or not oidia adhered to the legs and bodies of the flies when the flies were allowed to crawl a greater distance than in the above experiments, some flies were caused to go from a (−) mycelium on dung in a test-tube through a cardboard tube of slightly greater diameter and 3.5 feet long to a (+) mycelium in a test-tube at the other end of the cardboard tube. The flies were allowed to crawl over the (−) mycelium, and the other end of the cardboard tube was then held in the sunlight. The light attracted the flies to the (+) mycelium and they then walked over it. The flies were allowed to remain on each haploid mycelium only fifteen minutes. When the mycelia visited by the flies were examined four days later, they were found to have become diploid, while control mycelia remained haploid.

Further experiments were carried out with pairs of separate dung balls contained in each of the four large crystallizing dishes. One dung ball of each pair in a dish was inoculated with a *haploid mycelium*, while the other dung ball was left *uninoculated* and sterile. Into three of the dishes flies were introduced, but no flies were introduced into the fourth dish, which was kept as a control. At the end of one week it was found that in the control dish the uninoculated dung ball was still quite sterile, whereas in all of the three experimental dishes the uninoculated dung-ball had an oidial mycelium growing upon it. These results are easily explained, on the supposition that the flies in each dish carried oidia from the haploid mycelium of the one dung-ball to the surface of the other uninoculated dung ball and that the oidia there germinated and produced a mycelium.

One may ask: Do flies carry away from a haploid mycelium oidia and oidial fluid only or, in addition, are they able to carry away pieces of the mycelium? To solve this problem two separated dung balls were placed in each of the three large crystallizing dishes and were sterilized as in the first set of experiments in which flies were used (cf. Text-fig. 23). Then one of the dung balls was inoculated with a *diploid* mycelium and the other dung ball was left *uninoculated* and sterile. A diploid mycelium was employed in these experiments because it *does not produce any oidiophores or oidia*. After the inoculation of the dung-balls had been accomplished and the mycelium on the inoculated balls had grown over the dung sufficiently, some flies were introduced into two of the dishes. The other dish, into which flies were not introduced, served as a control. The flies were allowed to remain in the two experimental dishes for ten days.

At the end of fifteen days after the introduction of the flies into the experimental dishes neither in the experimental dishes nor in the control dish was there any mycelium growing on the uninoculated dung ball, and a similar result was obtained in another similar set of experiments made subsequently to the first set.

Had the flies broken off hyphal fragments from the diploid mycelium and deposited them on the uninoculated dung-ball doubtless these fragments would have grown and would have developed into vigorous mycelia; and the fact that, in the experimental dishes, the uninoculated dung balls remained free from mycelium is strong evidence that flies do not remove hyphal fragments from a mycelium. The experiments just described justify the conclusion that, when a fly visits a haploid mycelium, it carries away only oidia and oidial fluid and nothing else.

XI. THE OIDIA OF *COPRINUS LAGOPUS* COMPARED WITH THE PYCNIOspores OF THE RUST FUNGI.

The basidiospores of *C. lagopus*, under natural conditions, germinate in freshly-deposited horse-dung balls. The germ-tubes rapidly develop into haploid mycelia, and these haploid mycelia, some 2-3 days after the germination of the spores, develop oidiophores and oidia. The oidiophores project upwards from the substratum into the air and the oidia are set free into a drop of oidial fluid at the tip of each oidiophore. Even when haploid mycelia of opposite sex intermingle, they continue in the haploid state and produce oidia for 2-5 days after the germination of the spores (*vide* Section IV). The oidia are the first kind of spore to be produced on the young mycelia of *C. lagopus* and, sexually, they are haploid. In these respects they resemble the pycniospores of the Rust Fungi.

The oidia of *C. lagopus* are embedded in a fluid greedily sucked up by flies. This fluid therefore resembles the nectar which encloses the pycniospores of the Rust Fungi.

There can be no doubt that the function of the oidia of *C. lagopus* resembles the function of the pycniospores of such heterothallic Rust Fungi as *Puccinia graminis* and *P. helianthi*, i.e. the oidia, like the pycniospores, after being carried by flies from a mycelium of one sex to a mycelium of opposite sex, germinate and diploidize the mycelium of opposite sex on or near which they have been deposited.

In *C. lagopus*, just as in a heterothallic Rust Fungus, two mycelia of opposite sex, derived from two basidiospores of opposite sex, may (1) develop in contact with one another, or (2) develop far apart from one another. Let us consider how the sexual process is initiated in both these cases.

(1) Let us suppose that two haploid *C. lagopus* mycelia of opposite sex, say (*AB*) and (*ab*), derived from two basidiospores of opposite sex, have begun their development *close together* at or near the surface of a dung ball dropped in a pasture. As the mycelia grow in size they will meet and fuse and mutually diploidize one another; and the oidia will play no part in the diploidization¹ process. The same kind of thing happens in the heterothallic Rust Fungi; for, as Craigie has shown, in *P. graminis* or *P. helianthi*, when two mycelia of opposite sex, derived from two sporidia of opposite sex, develop close together in a leaf so that they come into contact with one another, even when the pycnial nectar containing the pycniospores is left undisturbed diploid aecia are produced under each mycelium.

(2) Let us now suppose that two haploid *C. lagopus* mycelia of opposite sex, say (*AB*) and (*ab*), derived from two basidiospores of opposite sex, have begun their development *far apart* at or near the surface of a single dung ball dropped in a field or one mycelium on one dung ball and the other mycelium on another dung ball. As the mycelia grow, owing to their distance apart it will be impossible for them to meet and fuse and mutually diploidize one another, and they will remain in the haploid condition unless and until flies or other insects carry (*AB*) oidia from the oidiophores of the (*AB*) mycelium to the (*ab*) mycelium and, vice versa, carry (*ab*) oidia from the oidiophores of the (*ab*) mycelium to the (*AB*) mycelium. When flies have thus transferred the oidia, the oidia will germinate and the oidial germ-tubes or mycelia will fuse with the mycelium of opposite sex to which the oidia have been brought and will diploidize it. In the heterothallic Rust Fungi, again, the same kind of thing happens; for, as Craigie has shown, in *P. graminis* or *P. helianthi*, when two mycelia of opposite sex, say (+) and (−), derived from two sporidia of opposite sex, are separated from one another on a single leaf or by one being on one leaf and the other on another leaf, so that they cannot intermingle, flies may transport (+) pycniospores from the pycnia of the (+) mycelium to

¹ For the origin of this term, *vide* A. H. R. Buller (5).

the (—) mycelium, and, vice versa, (—) pycniospores from the pycnia of the (—) mycelium to the (+) mycelium, with the result that diploid aecia are developed beneath each of the two formerly haploid mycelia.

The Hymenomycetes and the Uredineae both belong to the Basidiomycetes and are allied groups. The functional similarity of the oidia of *C. lagopus* and the pycniospores of the Uredineae is so great as to suggest the possibility that the oidia of the Hymenomycetes and the pycniospores of the Uredineae are related phylogenetically.

In one respect there is a marked difference between the oidia of *C. lagopus* and the pycniospores of the Rust Fungi. We know but little about the germination of the pycniospores, but it is certain that the germ-tubes or mycelia produced by pycniospores do not give rise to any further pycniospores. On the other hand, when oidia of *C. lagopus* germinate on sterilized dung-agar or horse-dung the mycelium derived from them soon produces a new crop of oidioophores and oidia; and, if these second-generation oidia are sown, the mycelium derived from them soon gives rise to third-generation oidia; and so forth. It may well be that, under natural conditions, when oidia are transported by flies to freshly-dropped dung balls, such oidial multiplication actually takes place.

In the Rust Fungi it seems certain that pycniospores, through the germ-tubes or mycelia to which they give rise, can cause diploid aecia to be developed only on mycelia of opposite sex derived from sporidia. As already recorded, in two experiments with *C. lagopus* where oidia of opposite sex were sown together in the same culture medium, the resultant mycelia of opposite sex failed to diploidize one another. Further experiments are needed to decide whether or not the results of these two experiments are normal. If they are normal, the mycelium derived from the oidia of *C. lagopus* is able to diploidize only those mycelia which have been derived from basidiospores, in which case the oidia behave like the pycniospores of the Rust Fungi.

Ráthay (17), in 1883, in Austria, identified 135 species of insects which visited the pycnia of various Rust Fungi; but, doubtless, a still greater number of species of insects visit recently-dropped horse-dung. The oidioophores and oidia of *C. lagopus* begin to appear on horse-dung two or three days after its deposition, and at this time insects can often be seen crawling over the dung in large numbers. It was noticed that, when fresh horse-dung is exposed on the laboratory table and *Drosophila* flies are about, the flies are soon attracted to the dung, doubtless by its odour. Under natural conditions in the open not only flies but many species of beetles, especially Staphylinidae and Scarabaeidae, frequent horse-dung; and in all probability these beetles, like flies, transport oidia from place to place. We may conclude that the insect agents required to carry the oidia of *C. lagopus* to mycelia of opposite sex are usually abundantly present in

pastures at the time when the haploid mycelia may be benefited by their services.

XII. SUMMARY.

1. The oidia of *C. lagopus* are produced on oidiophores which project away from the substratum into the air, and they are developed in, and are set free into, a drop of adhesive liquid which is of importance in the dissemination of the oidia.

2. The oidia of *C. lagopus* are produced only on haploid mycelia, never on diploid mycelia. On a mycelium derived from a spore oidia are produced only aerially or on the surface of the nutrient medium, never under the surface of the medium.

3. When many spores of *C. lagopus* of diverse sex are sown together in a culture medium, the haploid mycelia, in the first few days of their growth, produce oidiophores and oidia in abundance but do not fuse with one another. Then mycelia of opposite sex fuse with one another and mutually diploidize one another with the result that the production of oidiophores and oidia ceases.

4. Oidia are formed from the oidial branches at the apex of the oidiophore by: (1) the division of the protoplasm of the branch into two or three separate masses; (2) the shrinking or condensation of these masses with the formation of short water-filled gaps or spaces between them and at the base of the oidial branch; (3) the development of a wall at each end of each shrunken mass of protoplasm; and (4) the dissolution of the wall of the oidial hypha around the water-filled spaces.

5. The oidia of *C. lagopus*, under suitable conditions, germinate readily. Their germ-tubes, in culture media, develop into a mycelium which soon produces numerous oidiophores and oidia. By sowing oidia, twelve successive generations of oidia were obtained.

6. In *C. lagopus*, the mycelium developed from an oidium, as compared with a mycelium derived from a basidiospore, has thinner hyphae, produces more oidial fructifications per unit of surface, develops no aerial hyphae except oidiophores, grows more slowly, and remains sterile so far as ordinary fruit-bodies and fruit-body rudiments are concerned. Haploid mycelia derived from basidiospores, when grown separately on sterilized horse-dung, usually produce haploid fruit-bodies in ten to fourteen days, whereas a mycelium derived from oidia grew on sterilized horse dung for three months without any sign of fruit-bodies appearing.

7. Oidial fructifications of *C. lagopus*, associated with ordinary fruit-bodies of *C. lagopus*, were found on unsterilized horse-dung on four occasions. Thus, possibly for the first time, the oidia of a hymenomycetous fungus have been observed and recognized as such under natural conditions.

8. The oidia of *C. lagopus* are not transported by the wind; they may be scattered to a limited extent by rain, but they are mainly disseminated by coprophilous insects and, in particular, by flies.

9. Flies use the oidial drops on the oidiophores of *C. lagopus* as food. They carry away oidia on their feet and bodies, and they deposit oidia in a viable condition in their excreta.

10. From the mycelia of *C. lagopus* flies carry away oidial fluid and oidia only, and not any hyphae.

11. It has been proved experimentally for *C. lagopus* that, when flies walk on a (+) mycelium (mycelium of one sex) and then on a (−) mycelium (mycelium of opposite sex), they transfer (+) oidia to the (−) mycelium and (−) oidia to the (+) mycelium, with the result that both the (+) mycelium and the (−) mycelium become converted into diploid mycelia. The diploidization of haploid mycelia through the agency of flies took place when two mycelia were separated by a distance of 3.5 feet.

12. The oidia of *C. lagopus* very closely resemble the pycniospores of the Uredineae in their early appearance on haploid mycelia, in their being immersed in a fluid which is attractive to flies, in their transportation by flies from one mycelium to another, and in their ability to bring about the diploidization of mycelia of basidiosporous origin and of opposite sex.

The investigations recorded in the preceding pages were carried out in the Botanical Laboratory of the University of Manitoba. Professor A. H. R. Buller suggested the problem and gave generously of his time throughout the entire course of the work, and his stimulating criticism and helpful advice are gratefully acknowledged.

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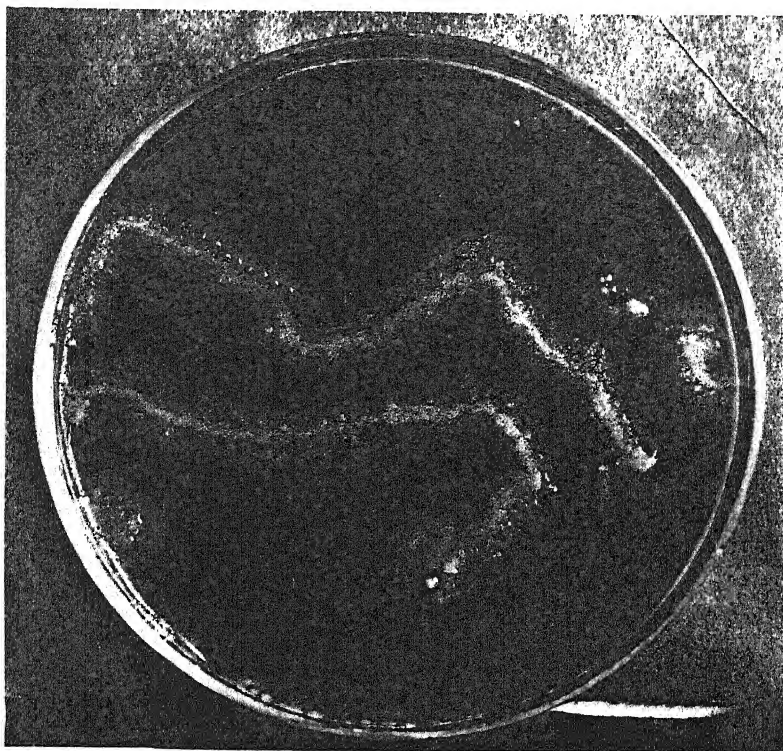
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EXPLANATION OF PLATE X.

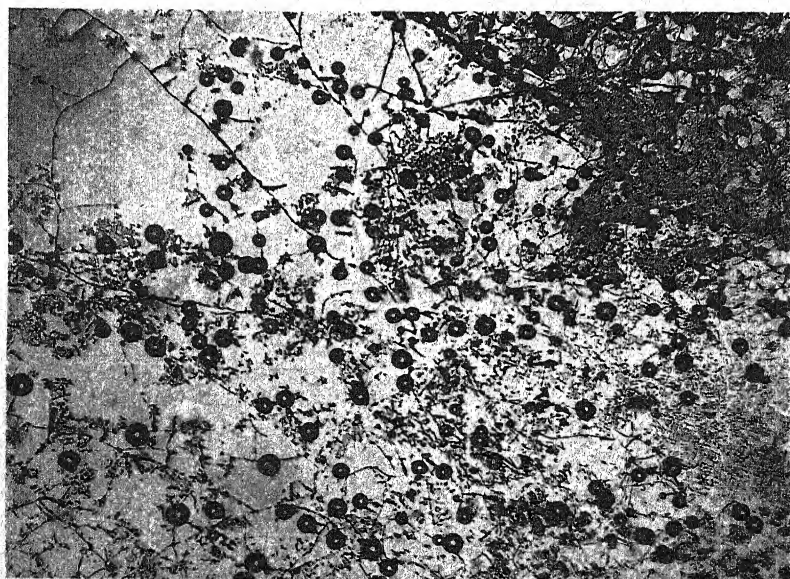
Illustrating Mr. H. J. Brodie's paper on the Oidia of *Coprinus lagopus* and their Relations with Insects.

Fig. 1. The dissemination of oidia of *Coprinus lagopus* by a fly (*Drosophila melanogaster*). A fly was allowed to walk for half a minute over a haploid mycelium (*ab*) contained in a Petri dish and then over a freshly-poured dung-agar plate where it made two main tracks with its body and feet. This second plate was photographed five days later, and the photograph is here reproduced. The fly deposited on the agar from its feet and body great numbers of oidia taken up from the mycelium (*ab*) and also bacteria with which it was contaminated before walking over the Petri dishes. The main tracks of the fly over the agar are now clearly marked out by bacterial colonies down the centre and mycelia growing away from the bacterial colonies on all sides. The mycelia originated from the oidia deposited by the fly, and they are already developing a new crop of oidiophores bearing the characteristic ooidal drops. By mating experiments it was found that the mycelia had the same sexual constitution, namely (*ab*), as the mycelium in the first of the two Petri dishes visited by the fly. Natural size.

Fig. 2. Photomicrograph of a haploid mycelium, No. 10 (*ab*), of *Coprinus lagopus*, to illustrate the production of oidia. The mycelium had grown for seven days in a hanging drop of malt-agar. The photomicrograph was then taken through the cover-glass and the culture medium. The drops, which appear as black balls, are all filled with oidia, and they are borne on the ends of oidiophores projecting into the air. The very numerous oidia in the film of moisture at the surface of the culture medium arrived at their present position: in part by ooidal drops coming into contact with the film of moisture, in part by being developed from the first on oidiophores lying in the film of moisture, and in part by floating about in the film of moisture. Magnification, 100.



1



2

Huth. coll.

A Cytological Investigation of *Stypocaulon scoparium* (L.), Kütz., with especial Reference to the Unilocular Sporangia.

BY

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With Plate XI.

THE work of Knight (3) on *Pylaiella littoralis* first showed that reduction division in the Phaeophyceae may take place in the unilocular structures which give rise to haploid zoids, and it has been shown (3 and 4) that these may either function as gametes (*Ectocarpus*) or as asexual zoospores (*Pylaiella*). That the unilocular sporangial mother-cell is the seat of reduction division in a member of the Sphacelariales also has been shown by Dr. Clint (1) for *Sphacelaria bipinnata*, in which the resulting zoids acted as gametes. In view of this work, another member of the Sphacelariales, *Stypocaulon scoparium* (L.), Kütz., was investigated, special attention being paid to the unilocular sporangia. With the exception of the case of a single plant referred to by Sauvageau (5) as bearing antheridia and oogonia, these unilocular sporangia are the only reproductive organs to have been described for this species.

The work was begun in the Botanical Department of the University of Liverpool, where it formed part of a thesis for the degree of Ph.D. A preliminary account of the results obtained was given at the meeting of the British Association in Glasgow in 1928. The investigation has since been continued in the University Department of Botany, Durham.

The material used for the cytological investigation was sent from Naples in a 4 per cent. solution of formalin after having been fixed in a chrom-acetic mixture of the following strength :

1 per cent. acetic acid.	.	.	20 cc.
1 per cent. chromic acid	.	.	25 cc.
sea water	.	.	55 cc.

Three supplies of material fixed in this way were received in December, 1926, and in October and December, 1927. On two occasions during the

early part of the present year (1930) supplies of fresh material have been received from Naples, through the courtesy of Professor Dohrn of the Zoological Station of Naples, to whom my best thanks are due.

Some difficulty was experienced in cutting the material owing to the presence of numerous diatoms which form an almost continuous layer over the surface of the plant. It was found, however, that a short treatment with a 10 per cent. solution of hydrofluoric acid removed all traces of silica and caused no appreciable contraction of the material.

The stains used were either Heidenhain's haematoxylin or brazilin, using iron alum as mordant in both cases. On the whole the haematoxylin method proved most satisfactory, although brazilin showed the structure of the chromatophores more clearly. Both orange G and picronigrosin have been used in combination with haematoxylin, the latter proving very satisfactory for protoplasm.

In some of the material, especially that fixed in December, 1927, the apical cells and sporangia contained a dense deposit of what is presumably an assimilation product whose nature has not been determined. This, especially in the older sporangia, masked the details of the cell structure, and it was found advisable to treat the sections for an hour or two with dilute hydrogen peroxide before mordanting and staining. This treatment renders the deposit colourless.

Various methods of clearing and staining unsectioned material were tried, in order to study the general morphology, &c. Chloral hydrate was found to have little clearing effect on the dense contents of the apical cells, and material treated with this reagent was found to be very difficult to stain. Sodium hypochlorite is very successful for showing up the structure of the dense stem-like portions of the plant, but in the more delicate parts its action is too strong and renders the nuclei almost indistinguishable. The following method has been found to be the most satisfactory, and it has been possible by its use to study the division of the nuclei in the apical and sub-apical cells, quite clearly in unsectioned material. After treatment with hydrofluoric acid to remove the siliceous matter, the material is washed and put into a solution of hydrogen peroxide (commercial strength) for two to four days. This effects an almost complete decolorization without bringing about any appreciable contraction in the cell contents. It is then washed in water and placed in a solution of acetic carmine for four days. After washing in a 5 per cent. solution of acetic acid it is treated with a solution of ferric ammonium citrate for half an hour, after which it is passed up through a series of alcohols and dehydrated in absolute alcohol. Although the material was extraordinarily resistant to high concentrations of hydrogen peroxide and hydrofluoric acid, some difficulty was experienced in finding a suitable clearing agent, since xylol, applied immediately after the absolute alcohol, also clove, cinnamon bark, and cajeput oils, all caused

very marked contraction of the protoplasm and collapse of the tissues. Aniline oil proved to be the most successful, although it produces a little contraction. The aniline is removed by xylol and the material transferred to very dilute Canada Balsam, which is allowed to concentrate gradually to a strength suitable for mounting. By the use of this method all stages in the division of the vegetative nuclei have been clearly seen.

General morphology.

The general morphology of the species has been previously described by many investigators and needs little further description here, but the remarkable power of regeneration of the apical cells may be mentioned. This is seen not only when injury to the original apical cell itself has taken place, but even when injury is very much more deeply seated in the older parts of the branches. Any cell, internal or cortical, below a break or injury seems to be capable of forming a new apical cell, and it is of common occurrence to see two or more cells growing out side by side, below an injury, each assuming the characters of an apical cell. Since, however, clusters of new branches are not commonly observed growing from a truncated branch, it would appear that eventually one cell only of the group develops.

Somatic mitosis.

The division of the vegetative nuclei has been previously studied by Swingle (9) and Escoyez (2), and the results obtained in the present investigation largely agree with theirs. Vegetative nuclear division has been studied principally in the apical and sub-apical cells which, owing to their large size, are ideal for the purpose.

In the apical cells the nuclei are large and embedded in protoplasm which stains very lightly and is very noticeably vacuolate. The chromatophores, which are small and ovate, are usually found in large numbers round the periphery of the cell, but they may also be found scattered throughout the cytoplasm. This scattering of the chromatophores usually takes place when their division, which is brought about by simple fission in the median plane, is taking place. In sections of the material in which there is a heavy deposit of the assimilation product, the individual chromatophores are frequently indistinguishable, and seem to be represented by deeply staining, somewhat wedge-shaped masses round the periphery of the cell. This is probably due to the fact that the assimilation product is being accumulated rapidly in the neighbourhood of the chromatophores which come to be embedded in it. In sections stained in brazilin the chromatophores appear heterogeneous, and in the cortical cells consist of lightly staining substance in which are embedded numerous deeply staining dots (Pl. XI, Fig. 2).

The nuclei vary considerably in size in different parts of the plant

according to the size of the cells which contain them. They are largest in the apical cells, and usually contain a single large, deeply staining nucleolus, although more than one is frequently present. Occasionally there occurs a second body which takes the stain differently and may be of the nature of a 'chromophilous spherule' as described by Lloyd Williams (10) for *Dictyota*. Sometimes the nucleolus appears homogeneous and may be lobed, but most frequently it is vacuolate. The vacuoles may be small and extremely numerous (Pl. XI, Fig. 1); sometimes there are two or three larger ones, and frequently a single vacuole may occupy a large proportion of the nucleus. In the nuclei of the lower cells of the plant, and even in those of the sub-apical cells, it is very common to find present numerous nucleoli of varying sizes.

The multinucleolar condition is interpreted by Escoyez (2) as representing the initial stages in the re-formation of the nucleolus after division from numerous deeply staining globules which will later coalesce. In the material here dealt with no connexion has been observed between this condition and the early stages after reorganization of the nucleus, and the multinucleolate condition has been seen to persist during the early stages of the next division, although this is most commonly the case in the nuclei of the unilocular sporangia.

The nucleus of the apical and sub-apical cells is large and is accompanied by a well-marked group of radiating astral filaments, amongst which are scattered small deeply staining granules. The deeply staining body figured by both Swingle (9) and Escoyez (2) at the central point of the centrosome has not been seen, although in one case there was present a large lightly staining body surrounded by a clear space from which the astral rays radiate (Pl. XI, Fig. 1). In the cells of the lower corticated regions the astral radiations are usually not so well marked, although they are sometimes distinctly seen.

The first indication that nuclear division is about to occur is the appearance of a second centrosome in connexion with the nucleus, but no evidence has been obtained of its mode of origin.

The spindle, when formed, is intranuclear, as figured by Swingle (9) and Escoyez (2), and in the apical and sub-apical cells may occupy only a small part of the nuclear cavity (Pl. XI, Fig. 2). In the early stages some of the spindle fibres project into the cavity of the nucleus and are not continuous from pole to pole, but later the spindle becomes more compact and definite in outline. Much of the deeply staining substance of the nucleus which precipitates out when division begins to take place remains within the cavity and does not take part in the formation of the chromosomes (Pl. XI, Fig. 2). This extra-chromosomal staining substance which remains within the nuclear cavity has always been found to be present as more or less definite granules as figured by Swingle, rather

than as the reticulate mass shown by Escoyez. The chromosomes, which first appear scattered over the spindle, range themselves round the periphery of the equatorial plate. They are small spheroid or discoid bodies, and they are so numerous that it is difficult to make a definite count, but their number is of the order of 30. Escoyez gives the chromosome number as being possibly 32. As will be seen, this number is confirmed by the haploid number given below for the nuclear divisions in the unilocular sporangia. Pl. XI, Fig. 2, shows a nucleus in the metaphase condition. After the split occurs the chromosomes move to the poles, each group still arranged in a circle round the periphery of the spindle. At the poles the chromosomes lose their individuality and merge into two lobed masses of chromatin. Throughout the foregoing stages the nuclear membrane persists, but in late telophase it disappears. When division is nearing completion the nuclei begin to reorganize and present the appearance of bars of staining substance crossing a colourless vesicle. At this stage traces of the spindle, which throughout the whole division is very clearly defined, can still be seen. Cell-wall formation does not take place until after the complete reorganization of the daughter nuclei, and it is very common to find binucleate cells in which there is, as yet, no trace of wall formation. When it finally occurs, it takes place by the deposition of deeply staining granules across the median plane of the cell between the two nuclei.

In the smaller cells of the lower part of the plant, even in the third or fourth cell from the apex, the spindle occupies the whole of the nuclear cavity throughout the division. Only one dividing nucleus has been found in the apical cell of a rhizoid, and in this the spindle, although intranuclear, occupies the majority of the nuclear cavity, and all the deeply staining substance appears to go to the formation of the chromosomes.

Unilocular sporangia.

The stalked unilocular sporangia may occur in groups of as many as fifteen, growing from the pad of tissue which occurs in the axil of each branch of the fertile axis. Even in the early mother-cell stage they contain numerous discoid chromatophores which appear heterogeneous, especially when stained with brazilin, although in the sporangia they do not appear so definitely spotted as in the vegetative cells, but frequently show barred markings (Pl. XI, Fig. 6).

The nucleus of the mother-cell is enlarged and usually contains a single well-marked nucleolus, although nuclei with two or three nucleoli are commonly seen. Only the prophase stages of the division of this nucleus have been found, but these have been sufficient to show beyond doubt that this is a heterotypic division, a fact which is confirmed by the chromosome number, obviously haploid, found in the nuclei during the subsequent divisions in the sporangium.

The chromatin of the mother-cell nucleus first appears as a thin discontinuous thread scattered throughout the nuclear cavity. This becomes a more definite spireme which is gradually concentrated to one side of the nucleus. Stages in synapsis are shown in Pl. XI, Figs. 4 and 5. Pl. XI, Fig. 4, shows a mother-cell in whose nucleus the chromatin thread has become thickened, appears beaded, and is arranged in very definite loops. At one stage the synaptic knot is very much condensed, the chromatin thread appearing as a tight spiral in connexion with the membrane at one side of the nucleus (Pl. XI, Fig. 5). Throughout the foregoing stages the nucleolus sometimes persists in a more or less stained condition, but in some nuclei it disappears early. In the later prophase stages the thread becomes broken up and the chromosomes begin to appear in pairs, but no diakinesis stage in which the thread has become completely broken up into paired chromosomes has yet been seen.

Although no further stages in the heterotype division have been seen, the binucleate condition resulting from it is common (Pl. XI, Fig. 6). These nuclei divide repeatedly, becoming smaller after each division. No centrosome has been seen in connexion with any of the nuclei of the sporangia. At an early stage the nuclei and chromatophores retreat to the periphery, where the subsequent divisions take place.

During the division of the smaller nuclei in the later stages of sporangial development, the spindle occupies all or nearly all the nuclear cavity, and the first prophase stages appear as in Pl. XI, Fig. 7, in which the chromatin is precipitated out as dots scattered throughout the nucleus. In the bigger nuclei of the young sporangium, however, division is intranuclear, and the first sign that it is about to take place is the contraction of the nuclear material from the membrane (Pl. XI, Fig. 8) and the gradual disappearance of the nucleolus. This central mass gives rise to the chromosomes and the spindle, the rest of the nuclear cavity containing little or no staining substance. In Pl. XI, Fig. 9, is shown an early stage in the formation of the spindle on which are scattered the chromosomes. At a slightly later stage, in which the spindle is fully formed (Pl. XI, Fig. 10), the chromosomes appear to be associated with the spindle fibres and are drawn towards the equatorial plate, where they form a rather compact mass in which it is difficult to distinguish the individual chromosomes in profile view (Pl. XI, Fig. 11). A plate view of the chromosomes in this stage is shown in Pl. XI, Fig. 12. During the metaphase the nuclear membrane begins to disintegrate (Pl. XI, Fig. 11), and subsequently the original limits of the nucleus are scarcely distinguishable.

Throughout these divisions the chromosome number has been found to be sixteen. Although the diploid number has not been definitely counted

it is obvious that the reduced number occurs after the reduction division in the unilocular sporangial mother-cell.

Division of the chromatophores also takes place in the developing sporangium, and when the divisions of the nuclei are completed a group of chromatophores becomes associated with each nucleus, the nuclei during the final stages of sporangial development being scattered throughout the cytoplasm of the sporangium (Pl. XI, Fig. 13). Cleavage of the protoplasm then takes place, and each nucleus, with its associated group of chromatophores embedded in a mass of cytoplasm, becomes differentiated into a spore (Pl., XI Fig. 14).

In some cases where spore release has evidently been delayed, the spores within the mature sporangium have been seen to be surrounded by a definite wall and germination has begun, *in situ*, by the division of the spores into two cells.

Innovation frequently takes place within the walls of the sporangia from which the spores have been shed. The stalk-cell immediately below grows up within the empty sporangium wall to develop into a new sporangial mother-cell. Sometimes certain of the stalk-cells may divide longitudinally so that the stalk is double. If the cell immediately below the empty sporangium has been divided in this way, both cells may project into the cavity (Pl. XI, Fig. 15), and both are capable of giving rise to stalked sporangia (Pl. XI, Fig. 16).

The behaviour of spores produced from the unilocular sporangia has not been observed. Several attempts have been made to grow fresh material sent by express post from Naples, still attached to portions of the substratum upon which it grows in nature, in an aquarium similar to that described by Scott (8). Although ripe sporangia were present, no spore release could be obtained, and none of the plants recovered from the effects of the journey from Naples, becoming discoloured a few days after introduction into the aquarium. The remarkable results of Dr. Knight for *Ectocarpus siliculosus*, showing that there may be complete difference in the life-history and behaviour between morphologically similar plants growing in different localities, suggests that a study of released spores from British plants would not be conclusive. It will therefore be necessary for the behaviour of spores from the Mediterranean plants to be studied *in situ*.

Sauvageau (6 and 7) grew cultures of spores from unilocular sporangia of *Stypocaulon scoparium* and described their early stages of germination. He observed no fusion of the spores, and refers to them throughout as zoospores. Since, however, he describes them as giving rise on germination, after the production of a multicellular disc, to branches of the normal vegetative type for *Stypocaulon*, it would appear that a study of the spore behaviour will be of great importance in establishing the life-history of the species.

A single plant has been described by Sauvageau (5) as bearing sexual organs. As the plant was a pressed specimen, however, the behaviour of the contents of the so-called sexual organs could not be followed, and it may be that they represented only abnormal forms of the unilocular sporangia. In the course of the present work sporangia have been seen, more particularly in cases where an accumulation of assimilation product is present, which give the appearance of having undivided contents even in an apparently ripe condition. These have proved on investigation to be either defective sporangia, in which the contents have begun to disintegrate, or else normal sporangia in which the configuration of the contents, when viewed in the unsectioned condition, has been masked by the deposit of assimilation product. It would appear possible that such sporangia have been described as oogonia, especially in view of the method of preservation of the specimen. Further evidence is thus necessary to establish the presence of such sexual organs in *Stypocaulon*.

SUMMARY.

1. The general morphology and the details of the vegetative nuclear division in *Stypocaulon scoparium* (L.) Kütz., have been found to be in close agreement with work already published. The chromosome number in the vegetative cells is 32.
2. The origin and development of the unilocular sporangia have been investigated and the cytology studied in detail.
3. The first nuclear division in the unilocular sporangial mother-cell is heterotypic, giving rise to nuclei with the reduced number of chromosomes, which throughout the subsequent divisions has been found to be 16.

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DESCRIPTION OF PLATE XI

Illustrating Dr. E. Marion Higgins' paper on a Cytological Investigation of *Stypocaulon scoparium* (L.), Kütz., with Especial Reference to the Unilocular Sporangia.

- Fig. 1. Resting nucleus with centrosome, from apical cell. $\times 1,200$.
Fig. 2. Heterogeneous chromatophores in cortical cells of material stained with brazilin $\times 600$.
Fig. 3. Metaphase in sub-apical cell showing deeply-staining granules in the nuclear cavity. $\times 1,900$.

Unilocular Sporangia.

- Fig. 4. Mother-cell with nucleus in synapsis. $\times 1,200$.
Fig. 5. Mother-cell nucleus in synapsis. $\times 1,200$.
Fig. 6. Binucleate stage. Heterogeneous chromatophores. $\times 1,200$.
Fig. 7. Prophases of the later divisions. $\times 1,200$.
Fig. 8. Early prophase of intranuclear division. $\times 1,200$.
Fig. 9. Spindle formation and early appearance of chromosomes. $\times 1,900$.
Fig. 10. Early metaphase. $\times 1,900$.
Fig. 11. Metaphase. $\times 1,900$.
Fig. 12. Plate view of metaphase. $\times 1,900$.
Fig. 13. Fully developed sporangium before cleavage of the protoplasm has occurred. $\times 1,200$.
Fig. 14. Fully developed spores in mature sporangium. $\times 1,200$.
Fig. 15. Innovation by two stalk cells of an empty sporangium. $\times 1,200$.
Fig. 16. Twin, stalked sporangial initials formed by the innovation of two stalk cells through an empty sporangium. $\times 600$.

Contributions to the Study of *Pyronema confluens*.

BY

H. C. I. GWYNNE-VAUGHAN

AND

H. S. WILLIAMSON.

With Plates XII-XIV and seven Figures in the Text.

INTRODUCTION.

PYRONEMA CONFLUENS, Tul., has been the subject of recurrent examination. Its morphology was first investigated by Tulasne Brothers (19) in 1861, then by de Bary (6) in 1863, by the Tulasnes again (20) in 1866, and by Kihlmann (12) in 1883. Biological and physiological studies were published by Kosaroff (13) in 1906, by Seaver (17) in 1909, and by Robinson (16) in 1926. The earliest cytological investigation was that of Harper (11) in 1900, in whose paper a full account of earlier work will be found; he was followed by Dangeard (5) in 1907, by Claussen (3) in 1912, by Brown (2) on the apogamous variety *inigeum* in 1915, and by Moreau and Moreau (15) in 1930. Tandy (18), in 1927, described the cytology of the closely related species, *P. domesticum*.

MATERIALS AND METHODS.

The material of the present research was found on a patch of burnt ground on the banks of Virginia Water in Windsor Great Park on October 19, 1929. Spores were collected, and germinated readily on nutrient agar in Petri dishes, giving rise to abundant fructifications. The presence of ascocarps in single spore culture showed the species to be monoecious and, in every sense of the word, homothallic. In order to obtain a just comparison with the work of earlier authors material was grown on Claussen's agar (8) and on the agar with powdered charcoal employed by Dangeard (5) and later by Moreau and Moreau (15), as well as on other media. It grew excellently on Claussen's agar, equally well on Barnes' agar made up without glucose but with inulin in the inner dish, and almost as well on Barnes' unaltered medium (9), ascocarps being obtained three or four days after the

spores were sown; on agar with charcoal, and lacking nutrient salts, the fruits were much less abundant and developed more slowly, appearing after five to seven days. Development was accelerated by incubation at 25° C. for the first twenty-four hours; with this treatment fruits appeared on Claussen's agar two days after sowing. As demonstrated by Robinson in 1926, light is essential for the formation both of the sexual organs and of the characteristic pink colour. Active material was fixed at all hours of the day and night, artificial illumination being used when sunlight was not available.

A number of fixing fluids was employed. For all but the youngest stages, from about the time of formation of the trichogyne onwards, by far the most satisfactory fixative was Flemming's strong fluid diluted with an equal quantity of water. During the early growth of the sexual organs, however, the density of food material made an osmic fluid useless; our best results at this stage were obtained with Merkel's fluid, as recommended by Harper (11). The young fructifications are surrounded by a mass of aerial hyphae, so that care is needed to induce them to sink in an aqueous fixing fluid. Further, the developing sexual organs and young ascocarps are lightly fastened to the substratum, and only too easily detached during fixation, washing, or passage through the alcohols. It is difficult to combine the vigorous pumping essential to good and rapid fixation with a treatment gentle enough to avoid the loss of valuable stages. After much experiment, we found an effective method was to prepare a number of pieces of coarse muslin about ten centimetres in diameter with a thread run round the edge so that each piece could be quickly drawn into a bag. The muslin was spread out, the agar was cut into blocks two or three centimetres across; these were quickly piled on the muslin, the fungus being untouched except where the knife had passed along the edges of the block, the thread was drawn, and the bag containing agar and fungus was dropped into a beaker of fixing fluid standing in a desiccator, to the lid of which the exhaust pump had been attached. The desiccator was now quickly closed, and the air pumped out until the material sank. The material remained in the muslin bag during washing and passage through the alcohols, and if not required for immediate use was stored in cylinders of Carberla's fluid, in which several bags could be placed one above the other. By these means the loss of young stages was greatly reduced. Material for embedding was taken through absolute alcohol and chloroform to paraffin with all precautions against disturbance, and was later cut into sections 7 to 10 μ thick.

Agar sections are apt to become detached from the slide; this was prevented by leaving the slides for twelve hours on the top of the water-oven, and by avoiding the use of wash bottles throughout the subsequent manipulation.

The Flemming material was bleached in alcoholic solution of chlorine and was stained chiefly in Heidenhain's haematoxylin. Sections were immersed for about twenty minutes in 4 per cent. solution of iron alum, washed in distilled water, placed for twenty-four hours in haematoxylin, and, after immersion in distilled water, washed out in iron alum. We found that it was advisable to make up a fresh solution of about 8 per cent. iron alum on each occasion by allowing a few crystals to dissolve in a Petri dish of distilled water to which three or four drops of glacial acetic acid had been added. Even with so strong a solution, the haematoxylin takes some time to wash out of fungal tissue; broadly speaking, the best picture is obtained with the most rapid washing out. The use of tap water, which is slightly alkaline, was avoided until washing out was complete. The sections were lightly counterstained with erythrosin in clove oil. For material fixed in Merkel's fluid we used, for the most part, a combination of gentian violet and light green (9, reprint). In good preparations the nuclei are purple, the walls a vivid green, and the cytoplasm almost colourless.

DEVELOPMENT OF THE GAMETOPHYTE.

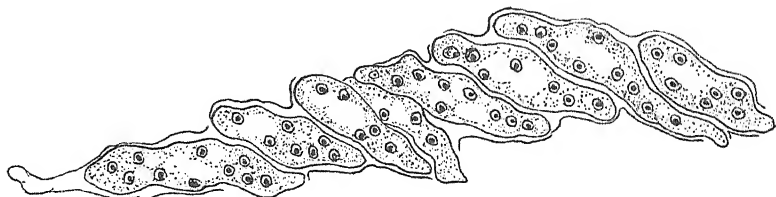
Under favourable conditions the spores will germinate in the ascus (Text-fig. 1) or at any time after they are shed. Germination has been obtained in spores twelve months old; they may survive for longer. Several nuclear divisions take place, each nucleus, once the spore has become multinucleate, dividing independently of its neighbours. Six chromosomes may be seen spread along the spindle in prophase (Pl. XII, Fig. 1), in the metaphase they are massed together, in the anaphase (Pl. XII, Fig. 2) six travel to each pole. After several nuclei have been formed, one or more stout germ tubes are put out (Pl. XII, Fig. 3); they branch freely, undergo septation, and give rise to a loose mycelium of narrow, coenocytic cells. Deeply staining granules are usually present on the transverse walls. At all stages there is marked variation in the size of organs, cell and nuclei, even though these be present in the same region and under apparently similar conditions.

The male and female organs are multinucleate from their first formation; several take part in the production of a single ascocarp (Text-fig. 2). They arise from separate hyphae which are often dichotomously branched. In respect of the morphology of the young organs we have nothing to add to the work of our predecessors.

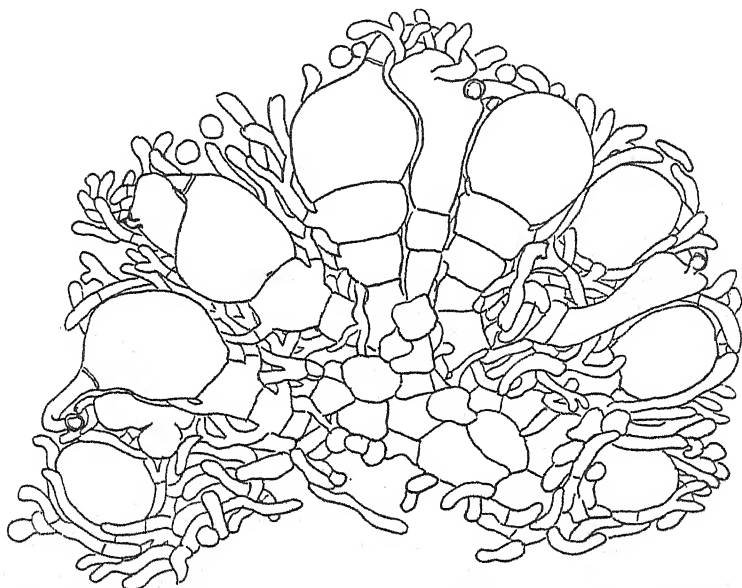
The antheridia are oblong or clavate (Pl. XII, Figs. 8, 12, 18, 20) or occasionally bifurcate (Pl. XII, Fig. 19), if basal walls have not separated the tips of the dichotomous branch. The number of nuclei counted ranged from 91 in a small antheridium to 203¹ in a fairly large specimen.

¹ The numbers were:—91, 120, 128, 141, 170, 203.

The young oogonia resemble the antheridia in form; they become globose at or soon after the time of fertilization. Each puts out a lateral or terminal process, the unicellular trichogyne, which is seen cut off by a



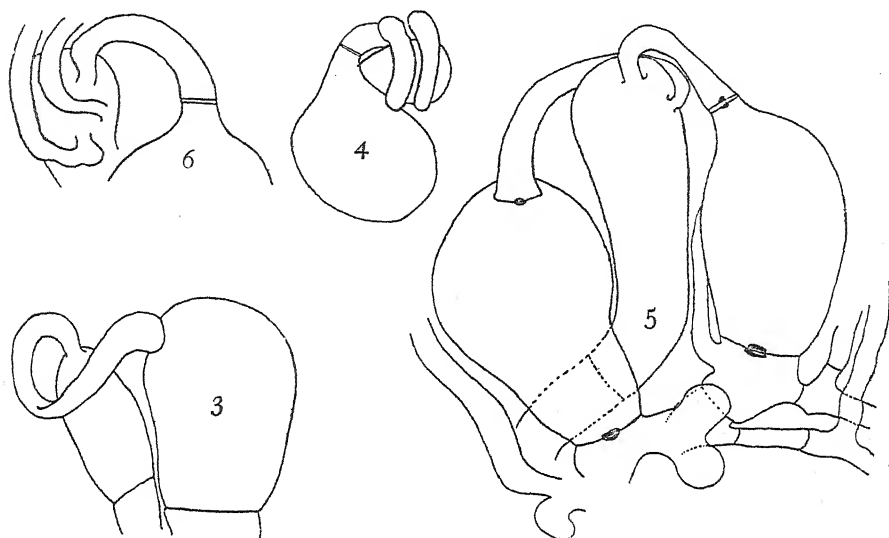
TEXT-FIG. 1. Germinating spores in an ascus; the two nuclei in the upper end of the third spore from the apex are in process of division. $\times 750$.



TEXT-FIG. 2. Section through ascocarp showing eight oogonia, after fertilization but before the formation of ascogenous hyphae. $\times 500$.

wall (Pl. XII, Fig. 7). As the sexual organs approach maturity the tip of the trichogyne becomes attached to an antheridium (Pl. XII, Fig. 8) and the separating walls disappear. Like the ripe antheridium, the oogonium at this stage contains, in round numbers, from 100 to 200 nuclei. This is in marked contrast to the condition in *Humaria granulata* (10), where the number of nuclei counted in the oogonium ranged from 1,408 to 593, and may doubtless be correlated with the fact that, in *Pyronema*, several antheridia and oogonia are concerned in the formation of a single ascocarp. The trichogyne is variable in length. It may reach an antheridium at some little distance, or may unite with one in contact with the oogonium; in the latter case it may be twisted (Text-fig. 3) or coiled round the antheridium (Text-

fig. 4). Not uncommonly two trichogynes (Pl. XII, Fig. 19, and Text-fig. 5), or even three (Text-fig. 6), become attached to the same antheridium, and presumably share the contents of the latter.



TEXT-FIGS. 3-6. 3. Oogonium with twisted trichogyne and attached antheridium. $\times 750$. 4. Oogonium with trichogyne coiled round upper part of antheridium. $\times 750$. 5. Antheridium to which trichogynes from two oogonia are attached. $\times 750$. 6. Three trichogynes in communication with one antheridium. $\times 750$.

When continuity is first established between the antheridium and trichogyne, the wall at the base of the latter is still intact, though it is thick enough (Pl. XII, Fig. 8) to suggest that gelatinization has begun. A little later a rounded aperture is formed (Pl. XII, Figs. 9, 10) in the neighbourhood of the large metaplasmic granule lying near the middle of the wall, and finally, as the male nuclei pass into the oogonium, the whole wall appears to be swept away or pressed against the sides of the oogonium (Pl. XII, Figs. 11, 12). In Pl. XII, Fig. 11, a trace of the wall is still visible between the two nuclei on the outer side of the curve. The wall between the oogonium and trichogyne is soon reformed; the whole process, indeed, is rapid, continuity between oogonium and trichogyne being found only in material growing under good conditions which has been quickly fixed without preliminary disturbance. Like other critical stages it is doubtless entered upon only in healthy material, and, once begun, is completed unless the organ is killed too quickly for this to occur.

Moreau and Moreau have called attention to the danger of mistaking for open trichogynes those occasional ascogenous hyphae (Pl. XII, Fig. 16) which possess a relatively wide region of attachment to the oogonium. Having regard to the age of the oogonium when the ascogenous hyphae

are given off and to the staining capacity of the trichogyne wall, this difficulty may be overcome; a more probable source of error lies in the position of the basal wall, which is sometimes a long way up the trichogyne (Pl. XII, Fig. 17), so that sections showing only the lower part of a closed trichogyne may give the impression that it is open.

After the fertilization stage the antheridium contains vacuolate cytoplasm and a number of nuclei.¹ These are at first quite distinct (Pl. XII, Fig. 18, right hand antheridium), but soon their outlines become difficult to determine (Pl. XII, Fig. 18, left hand antheridium), and they pass by gradual stages (Pl. XII, Fig. 19) to the condition of deeply staining debris (Pl. XII, Fig. 20, Pl. XIII, Figs. 21, 22). No doubt some antheridia fail altogether to function; both antheridia and oogonia full of disintegrating contents are sometimes to be found in older ascocarps.

While the processes of fertilization are taking place, vegetative hyphae have grown up around and between the sexual organs, forming the first stage of a loosely arranged sheath (Pl. XII, Fig. 20, Pl. XIII, Fig. 21). They have dense contents and few transverse septa; their resting nuclei are pale and rather difficult to see, but the nuclear figure becomes very clear in mitosis (Pl. XII, Figs. 4, 5, 6) and shows six chromosomes (Pl. XII, Fig. 4) in the prophase of division. All nuclei in a branch divide simultaneously, those nearer the apex being somewhat in advance of those towards the base. Six chromosomes were also counted in the nuclei of the paraphyses.

DEVELOPMENT OF THE SPOROPHYTE.

At the time of entry of the male nuclei, the nuclei of the oogonium lie around the walls (Pl. XII, Figs. 11, 12) or are grouped especially at the base of the trichogyne and just above the stalk cell (Pl. XII, Fig. 10). In no case did we observe the massing of nuclei in the middle of the oogonium recorded by Harper at this stage. It is hardly surprising to find some variation in material collected from habitats so remote as Lake Forest and Windsor Park.

After the male nuclei have entered, nuclei in the oogonium are seen to lie in pairs (Pl. XII, Fig. 13), and fusion soon follows (Pl. XII, Figs. 14, 15), all fusions in a given oogonium taking place at nearly the same time. We were fortunate in obtaining clear preparations from material which had been growing in sunshine up to the moment of fixation; in many of the oogonia nuclear fusion was in progress, and, as may be seen by a comparison of Pl. XII, Figs. 14 and 15, with Pl. XIV, Fig. 50, showed the same character as the subsequent fusion in the ascus. At the time of nuclear fusion there is no sign of ascogenous hyphae, another minor variation

¹ The numbers were:—41, 58, 59, 59 (an antheridium to which two oogonia were attached); 62, 66, 67, 82, 116 (an antheridium which showed no trichogyne, and may have failed to function).

between our material and that of Harper. Ascogenous hyphae, however, appear soon after (Pl. XIII, Figs. 21, 22), and nuclei flow into them in an almost continuous stream. There is at this time no suggestion of a paired arrangement, odd and even numbers in the groups of nuclei being equally common.

The ascogenous hyphae are usually narrow where they leave the oogonium (Pl. XIII, Figs. 23, 24), allowing the passage of only one nucleus at a time. The free portions are swollen (Pl. XIII, Figs. 22, 24, 25); soon they give rise to one or more relatively narrow branches (Pl. XIII, Figs. 24, 25), in which the nuclei lie in single file. As in *H. granulata* (19) karyokinesis takes place simultaneously at least once in all nuclei of a group of ascogenous hyphae and also in the nuclei still in the oogonium (Pl. XIII, Figs. 25, 26). Division is rather more advanced in nuclei remote from the oogonium than in those in or near it, suggesting that the impulse to divide originates at the tips of the developing hyphae. Such simultaneous mitosis, which affects a variable number of nuclei within a communicating mass of cytoplasm, but in which the divisions are independent and do not exactly synchronize, must be distinguished from the conjugate division of pairs of nuclei, the spindles of which lie parallel in the telophase.

In *H. granulata* mitosis at this stage affects every nucleus in the oogonium; in *Pyronema*, on the other hand, a varying number of oogonial nuclei remains in interphase. It will be recalled that *Humaria* is apogamous, fusions taking place between oogonial nuclei, so that practically every nucleus may be expected to fuse. Here, on the other hand, fusions are presumably between nuclei of different sex, and some superfluous, haploid nuclei may be expected to remain. We are inclined to infer that the nuclei in interphase during mitosis in the oogonium and its attached ascogenous hyphae are such superfluous female nuclei; they have not been seen in the ascogenous hyphae. Their numbers vary. A large oogonium showed 34 nuclei dividing and 66 in the resting state; in a smaller specimen 35 were dividing and 21 in interphase, while the smallest examined showed only 3 resting nuclei and 76 in division. The number of superfluous nuclei, as might be expected, was largest in the largest organ. These numbers do not include the nuclei dividing in the ascogenous hyphae, which are difficult to count at so late a stage, as the branches cannot certainly be followed from one section to another.

A curious feature of *P. confluens* may here be noted. All critical stages—the mitoses in the spore, the passage of male nuclei into the oogonium, their fusion with the female nuclei, the mitoses in the oogonium and its hyphae—may long be sought in vain. In material in which they appear, however, they are of frequent occurrence, numbers of cells or organs being at almost the same stage of development. It would seem that, when

such a stage is approached, the development of the cell or organ is delayed until it receives the stimulus of some necessary external condition. We have reason to suspect that, in some cases at any rate, sunshine may be such a factor.

It has already been said that the number of nuclei in the oogonium is in general terms between 100 and 200. Careful counts have been made in eighteen oogonia, which could be traced in their entirety, before fertilization, and in the same number both during and after that stage. The results are given in Table I. In the case of oogonia with ascogenous hyphae, the nuclei in the latter were counted as well as those in the oogonium, the stage selected being that while the branches were still short and before mitoses had taken place. The nuclei in these oogonia include both diploid nuclei and such haploid nuclei as had failed to fuse.

TABLE I.

	Number of Oogonia.	Smallest number of Nuclei.	Largest number of Nuclei.	Average number of Nuclei.
1. Oogonia before entry of male nuclei ¹ .	18	98	201	150
2. Oogonia with fusing nuclei ² .	18	182	393	276
3. Oogonia with ascogenous hyphae ³ .	18	96	211	163
4. Difference between 1 and 2.	—	84	192	126

These figures indicate an increase in the number of nuclei in the oogonium at the time of fertilization and a subsequent decrease to about the original figure. There is no suggestion till a much later date of disintegrating nuclei in the oogonium, and, even apart from more direct evidence, it may be inferred that the increase is due to the entrance of male nuclei and the following decrease to fusion. The number of nuclei which fuse varies widely according to the relative size of the associated male and female organs, the number of trichogynes which connect with one antheridium, and the number of nuclei left behind in the antheridium to disintegrate. We did not see any examples of oogonia with double trichogynes, but a branched trichogyne in contact with two antheridia was figured by Harper, who frequently found two antheridia applied to the surface of a single oogonium. There seems no reason that both should not function in such a case; it offers another possibility of variation. The number of nuclei in interphase in oogonia with dividing nuclei, though

¹ The numbers were:—98, 99, 108, 108, 134, 142, 147, 154, 154, 158, 166, 172, 173, 180, 198, 201, 201.

² The numbers were:—182, 205, 209, 211, 235, 236, 241, 251, 256, 259, 276, 278, 282, 287, 293, 308, 368, 369, 380, 393.

³ The numbers were —96, 114, 131, 137, 147, 147, 150, 156, 161, 171, 173, 183, 186, 197, 200, 203, 211.

known in too few cases for useful generalization, also suggests that the number of male and female nuclei in the oogonium may sometimes be nearly equal, sometimes widely different. It would appear, then, that most of the female nuclei may undergo fusion, or a considerable proportion may remain unpaired; and that a considerable proportion of the male nuclei may remain in the antheridium. Possibly this state of affairs may have led to the condition in *P. domesticum*, where Tandy (18) found both diploid and haploid nuclei in the cells of the ascogenous hyphae. We have, however, seen no evidence of the haploid number of chromosomes among the mitoses in the sporophyte of *P. confluens*.

After the division already described in the nuclei of the oogonium and attached ascogenous hyphae (Pl. XIII, Figs. 25, 26, 27), the sister nuclei may lie for a time side by side (Pl. XIII, Fig. 28), an arrangement especially likely to occur when the spindle has lain at an angle to the long axis of the branch. As the branch elongates the nuclei tend to move apart, those in the upper reaches becoming more or less evenly distributed (Pl. XIII, Fig. 28), though there may be some crowding among those moving up from below (Pl. XIII, Fig. 29). The tips of the hyphae at this stage are rounded, full of contents, and with every appearance of active growth. No walls are formed, but elongation soon results in the formation of tracts almost empty of cytoplasm, separating the branches of the ascogenous hyphae from one another and from the oogonium, so that, from henceforth, divisions in each proceed independently. Pl. XIII, Fig. 30, shows a hypha with three nuclei in mitosis, while a region empty of cytoplasm may be discerned at the base. In Pl. XIII, Fig. 31, one of the nuclei from a similar hypha appears in anaphase; in Pl. XIII, Fig. 32, the nuclei nearest the tip are already in telophase, while in one lower down a late anaphase is seen. As in the earlier divisions in the ascogenous hyphae, and in those of the sheath, there is a noticeable lag from apex to base. The tips of the hyphae are now less rounded and have a less active appearance than those of the younger filaments, and in many cases the upper end of the hypha is curved, so that part lies parallel to the surface of the hymenium.

As recorded by Claussen (3) the most striking character of the ascogenous hyphae at a later stage is the presence of a series of binucleate cells (Text-fig. 7). He inferred, not unnaturally, that these nuclei were derived from the pairs of nuclei which may be seen (Pl. XIII, Fig. 28) at an earlier stage; he assumed them to be male and female and to undergo conjugate division. A study of the intervening stages, however, and especially of the mitoses concerned, has led us to a different conclusion. In *P. confluens*, as in other members of the Ascomycetes, the mitotic spindle is intranuclear, and the nuclear area of the dividing nucleus is visible as a well-marked vacuole (Pl. XIII, Fig. 32). It persists between the daughter nuclei, so that they move apart, and the daughter nuclei of adjacent

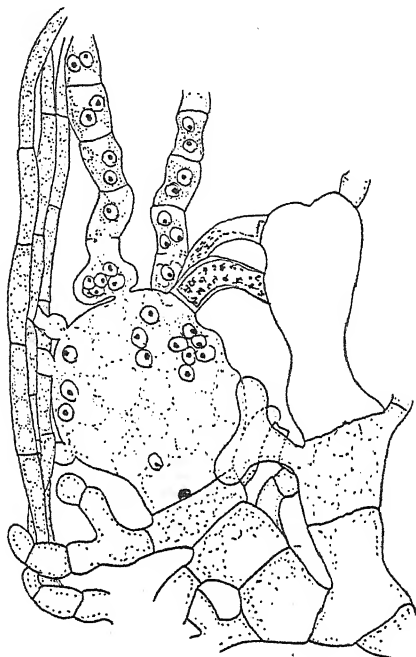
telophases are brought near together (Pl. XIII, Fig. 33), or even into contact. They must often be of the relationship of first cousins, since, as stated above, sister nuclei are apt to lie next to one another in hyphae at the stages shown in Pl. XIII, Figs. 28, 29. The distal daughter nucleus of the distal telophase is isolated in the tip of the hypha (Pl. XIII, Figs. 33, 34, 35). Walls, the first to appear in the ascogenous hyphae, are formed between the sister nuclei, across the regions formerly occupied by the spindles, and the remains of the old nuclear vacuole can be seen for a long time (Pl. XIII, Figs. 36, 37, 38, 39) persisting on either side of the wall. The development of the wall appears to be annular; an attempt has been made to indicate this in Pl. XIII, Fig. 36. Here the lowest wall is fully formed, the next can be seen both in high and low foci, but in the middle focus a clear space is observed. The positions of the upper walls are indicated, as in previous figures, merely by some thickening of the cytoplasm. As a result of the formation of walls between sister nuclei, the distal nucleus is cut off alone, and each hypha has a terminal, uninucleate cell, while the proximal cell, where the hypha can be traced throughout its length, is always found to contain either one nucleus or three, according to whether the formation of the lowest wall has taken place. A comparable state of affairs has long been recognized in the older regions of the ascogenous hyphae, where all investigators agree that the crozier, after simultaneous division of its nuclei, has a uninucleate, terminal cell, a binucleate, penultimate cell, and a uninucleate stalk. The arrangement is exactly the same in short ascogenous branches (Pl. XIII, Fig. 39), in which the division of only two nuclei has occurred. More commonly three, four, or five nuclei are present and divide at the same time, so that a row of several binucleate cells is seen (Pl. XIII, Figs. 36, 37, 38) between the uninucleate tip and the proximal cell.

As already stated, the ascogenous hyphae are usually bent, so that their upper parts lie more or less transversely along the subhymenium (Pl. XIII, Figs. 36, 37, 38, 39). The binucleate cells now begin to bud out, each forming a short, binucleate branch (Pl. XIII, Figs. 36, 37, 38, 39), which curves (Pl. XIII, Figs. 40, 41, Pl. XIV, Figs. 48, 49) to produce the familiar crozier. Since it has been suggested by Claussen (3) and others that the crozier originates, like the clamp connexion of the Basidiomycetes, by the development of a lateral bulge, we have made a special examination of the young stages (Pl. XIII, Figs. 40, 41) and are satisfied that the crozier is produced, as stated by Dangeard (4), Harper (11), and others, by the growth and curvature of the tip of the branch; like the earlier bend in the ascogenous branches, this has the advantage of bringing into a favourable position the cell next to grow out, in this case the ascus.

While the young crozier is usually binucleate, occasional trinucleate (Pl. XIV, Fig. 42) or tetranucleate examples are found; in such cases all

nuclei in the crozier divide, as usual, at the same time (Pl. XIV, Fig. 43). We have found no evidence with regard to their subsequent history.

Harper, to judge from his Fig. 31, must have seen stages very similar to our Pl. XIV, Fig. 49. The condition shown in this and earlier figures,



TEXT-FIG. 7. Two oögonia, one of which still retains some contents and has given rise to ascogenous hyphae. The latter are of the small type seen in Fig. 24; they show binucleate cells. $\times 750$.

with a small, non-effective, terminal cell, is indicated by Claussen in his Figs. 35 to 40, but he draws the terminal cell with two small nuclei. Since the nuclei are often granular (Pl. XIII, Figs. 33, 34, 37, 38), and since the remains of a vacuole are present (Pl. XIII, Figs. 37, 38, 39) near the transverse wall, it is sometimes difficult to be sure of the number of nuclei; but we have never found a case where there were certainly two, while cells with an undoubted single nucleus are to be found in every section. Maire (14), in 1905, recorded the occurrence of binucleate cells in the ascogenous hyphae of various fungi, and Guilliermond (8), in the same year, called attention to the ascogenous hyphae of *Peziza catinus*, each of which arises from a multinucleate cell and consists of a row of binucleate cells, ending in a distal cell which is uninucleate. This is precisely the arrangement in *P. confluens*, but the binucleate cells of *Peziza catinus* give rise at once to asci, instead of forming croziers. It is possibly a more primitive arrangement.

In the ascogenous hyphae Claussen, in his Figs. 42 and 43, has shown

conjugate mitosis, the two nuclei beginning to divide one behind the other, but later, as their spindles elongate, lying side by side with spindles parallel to the long axis of the filament. We have made considerable search for these telophases, but we have found them only in the crozier, where they are shown in Pl. XIV, Figs. 47, 48. Such stages, if viewed, like Pl. XIV, Fig. 47, from an angle which conceals the bend of the crozier, might easily be interpreted as belonging to a conjugate division, though the nuclei actually lie one behind the other in the curved filament.

Both in the young ascogenous hyphae and in the croziers it is possible to count the number of chromosomes. As demonstrated by Claussen, there are twelve chromosomes in the prophase (Pl. XIV, Fig. 44), and a considerably larger number can be seen in the anaphase (Pl. XIII, Fig. 31), which, in favourable cases, when the hyphae and nuclei are large and clear, can be recognized as twenty-four, twelve going to each pole (Pl. XIV, Fig. 45). Since six chromosomes are present in the nuclei of the spores and of the cells of the sheath, twelve is clearly the diploid number, and indicates that sexual fusion has taken place before the formation of the ascogenous hyphae.

DEVELOPMENT OF THE ASCUS.

In the subterminal cell of the crozier two diploid nuclei fuse (Pl. XIV, Fig. 50) to constitute the tetraploid, definitive nucleus of the ascus. The ascus grows up, the definitive nucleus enlarges, and it passes into the prophase of meiosis.

At the time of formation of the spindle, twelve gemini can be seen, and, at the close of the heterotype division, twelve chromosomes pass to each pole (Pl. XIV, Fig. 51). This number was recorded by Claussen, and Harper and Dangeard also saw about ten, the underestimate being doubtless due to the difficulty of counting a large number of these minute bodies.

In the metaphase of the second division in the ascus six chromosomes are seen on the spindle (Pl. XIV, Fig. 52), and six pass to each pole. Six are present throughout the third division (Pl. XIV, Fig. 53), so that the nuclei around which the spores will develop each contain six chromosomes. Harper did not make a detailed study of the second and third divisions of the ascus; Dangeard noticed, and was puzzled by the smaller number, which he estimated as four or five. He was satisfied with the accuracy of his observation, for, as he pointed out, 'nous ne voyons pas d'où viendrait une erreur de moitié dans la numération'.

Brown (2), in the apogamous variety *inigerum*, found five chromosomes in all divisions, including those in the germinating spore, so that here also there is a small haploid number. Claussen described twelve chromosomes in the second and third divisions, but he did not figure this number, and

indeed showed both a second metaphase (his Figs. 105, 106) and a third telophase (his Fig. 117), in which no larger number than six can be counted.

There is thus general agreement that a large number of double chromosomes, twelve according to Claussen and ourselves, ten according to earlier authors, is present in the first meiotic prophase, and we are able to confirm Dangeard's observation, with which the evidence of Claussen's figures is also in accord, that the number of chromosomes is half as great in the second and third divisions in the ascus as in the first.

In *P. confluens*, then, the haploid number, observed in the germinating spore, in the cells of the sheath and in the paraphyses, is six; the diploid number, seen by Claussen and ourselves in the ascogenous hyphae, is twelve; the twelve gemini seen by Claussen and ourselves in the meiotic prophase represent twenty-four chromosomes, the tetraploid number, and it is in fair accord with the number seen by Harper and Dangeard. In the anaphase of the first meiotic division twelve chromosomes are seen going to each pole, and the daughter nuclei formed are diploid. Finally a second reduction, achieved in the remaining divisions in the ascus, brings the number of chromosomes to six, and the haploid condition is restored.

The apogamous variety, *inigum*, in which Brown counted five chromosomes both in the ascus and in the spore, has clearly lost all trace of sexual fusion, and is haploid until the fusion in the ascus, when reduction at once supervenes.

Tandy (18) was able to count seven chromosomes in the germinating spore of *P. domesticum*. He found fourteen in the ascogenous hyphae when sexual fusion had taken place and seven when it failed; there were fourteen or seven gemini in the meiotic prophase, but always seven chromosomes in the third division in the ascus.

Some interesting speculation is opened by the occurrence of five, six, and seven as the haploid numbers of chromosomes in these nearly related forms.

We are in agreement with all Harper's observations, except that, in our material, there is no massing of the female nuclei in the middle of the oogonium, and the ascogenous hyphae are somewhat slower to develop.

We are in agreement with the observations of Dangeard and of Moreau and Moreau, and, like them, we have found male nuclei disintegrating in the antheridium, but, in addition, we have seen the entry of male nuclei into the oogonium and their fusion there with the female nuclei; we found such stages only in active material which had been rapidly fixed. We are in agreement with Dangeard in his observation of the change in the number of chromosomes between the first and the second and third divisions in the ascus.

We are in agreement with Claussen in respect of the passage of male nuclei into the oogonium and their association with the female nuclei in pairs, but we have seen the process completed by the fusion in the oogonium of these nuclei. We agree with Claussen's observation that the ascogenous hyphae have binucleate cells, but, by a study of their development, we have shown that these nuclei are the daughters of neighbouring telophases and are often of the relationship of cousins. All our evidence is against the hypothesis that they are the result of conjugate division of paired but unfused male and female nuclei. We can confirm Claussen's statement that the number of chromosomes in the nuclei of the ascogenous hyphae and the number of gemini in the first division of the definitive nucleus of the ascus is twelve; but we have shown that this is the diploid number, the haploid number in the germinating spores and in the cells of the sheath being six. The definitive nucleus of the ascus, since it contains the diploid number of gemini, is itself tetraploid, and meiosis is followed by a second, or brachymeiotic (11) reduction.

Since *P. confluens* is neither dioecious nor in any other sense heterothallic, the near relationship of the nuclei which fuse in the ascus is without general significance. It will be of interest to ascertain whether such a relationship exists where male and female organs are borne on different plants or where fructifications occur only on a dithallic mycelium. In one case of the latter type, *Neurospora tetrasperma*, Moreau and Moreau (15) have already recorded a relationship of first cousins between the nuclei of the young ascus.

SUMMARY.

1. The spores of *Pyronema confluens*, Tul. germinate without a period of rest. Fertile ascocarps are produced in single spore culture.
2. At maturity the antheridia and oogonia contain from one hundred to two hundred nuclei each. The unicellular trichogyne effects contact with the antheridium, and the walls between antheridium and trichogyne disappear.
3. The wall separating the trichogyne and oogonium swells; a hole is formed in its centre; the wall disappears as the male nuclei pass into the oogonium. It is soon reformed.
4. Both antheridia and oogonia may fail to function. In functional antheridia anything up to half the nuclei may remain behind and degenerate *in situ*.
5. The male and female nuclei fuse in pairs in the oogonium. Nuclear fusion was seen in progress; the number of nuclei in the oogonium was counted before, during, and after fertilization; the number of chromosomes was counted before and after fusion. The nuclei fuse in the resting state.
6. After fertilization ascogenous hyphae grow out from the oogonium;

they branch; nuclei pass into them in single file; a simultaneous division takes place in the diploid nuclei of the oogonium and its branches before the latter are cut off.

7. As the branches of the ascogenous hyphae elongate they become separated one from another by tracts empty of cytoplasm; the nuclei in each branch lie in single file; they divide simultaneously; when mitosis is complete the daughter nuclei are separated by a vacuole derived from the nuclear vacuole of the parent nucleus; the cell wall, which is annular in development, is formed across the vacuole. As a result, the distal and proximal cells of the hypha contain each a single nucleus; the intervening cells are binucleate; their nuclei are often of the relationship of cousins.

8. The binucleate cells bud out; each bends over to form a crozier from the penultimate cell of which the ascus is produced; its development follows the usual course.

9. The number of chromosomes in the germinating spore, in the cells of the sheath, in the paraphyses, and in the second and third divisions in the ascus is six, the haploid number. The number in the ascogenous hyphae, which constitute the sporophyte, is twelve. After the fusion in the ascus the first meiotic division shows twelve gemini. The definitive nucleus of the ascus is thus tetraploid. A second reduction takes place in the second and third divisions in the ascus.

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EXPLANATION OF PLATES XII-XIV.

Illustrating Professor Dame Helen Gwynne-Vaughan and Mrs. Williamson's paper
on *Pyronema confluens*.

PLATE XII.

Fig. 1. Prophase in one of the nuclei of the germinating spore, showing six chromosomes, the haploid number, spread along the spindle. $\times 2,600$.

Fig. 2. Anaphase of same, six chromosomes travelling to each pole. $\times 2,600$.

Fig. 3. An older spore with one nucleus in telophase. $\times 1,000$.

Fig. 4. Part of a hypha of the sheath with two nuclei in prophase, each showing six chromosomes. The position of this hypha is shown in Fig. 22. $\times 2,600$.

Fig. 5. Tip of a vegetative hypha with nucleus in metaphase. $\times 2,600$.

Fig. 6. A branching hypha of the sheath; the distal nucleus is already in early telophase. $\times 2,600$.

Fig. 7. A young oogonium with the trichogyne cut off by a basal wall. $\times 1,000$.

Fig. 8. Two antheridia arising from a dichotomous hypha; a trichogyne is in contact with each. $\times 1,000$.

Fig. 9. Two oogonia borne on a dichotomous branch; the trichogyne of one of them is cut across in two places, exposing the basal wall in surface view; it shows a large central opening. The dotted lines indicate the curve of the trichogyne in the plane at right angles to that of the paper. $\times 1,000$.

Fig. 10. An oogonium with a basal wall open as in Fig. 9, but seen laterally. $\times 1,000$.

Fig. 11. Transverse section of an oogonium with an open trichogyne; the rim of the basal wall is just visible on the outside of the curve as a small, light area between two nuclei. $\times 1,000$.

Fig. 12. Surface section of an oogonium with an open trichogyne; the nuclei, which are lining the wall, as in Fig. 11, are seen in surface view. 12 a shows the size of this oogonium in median section. $\times 1,000$.

Fig. 13. Part of an oogonium after the entry of the male nuclei; the sexual nuclei lie side by side. $\times 2,600$.

Fig. 14. An oogonium showing fusion in pairs of the sexual nuclei. $\times 1,600$.

Fig. 15. Nuclear fusion in the oogonium. $\times 2,600$.

Fig. 16. A young ascogenous hypha with an unusually wide base, for comparison, apart from the branch in a lower focus, with a trichogyne. $\times 1,000$.

Fig. 17. Part of an oogonium with the wall some way up the trichogyne. $\times 1,000$.

Fig. 18. Two antheridia after fertilization; one shows vacuolate cytoplasm and scattered nuclei, in marked contrast to the crowded condition in the antheridia of Figs. 8 and 12; in the other disintegration has begun. $\times 1,000$.

Fig. 19. A bifurcate antheridium in which disintegration is further advanced ; a trichogyne is attached to each fork. $\times 1,000$.

Fig. 20. A pair of antheridia at a somewhat later stage. $\times 1,000$.

PLATE XIII.

Fig. 21. An oogonium putting out ascogenous hyphae, the trichogyne and antheridium are in process of disintegration. $\times 1,600$.

Fig. 22. A rather older stage ; in a vegetative hypha near the antheridium are seen the mitoses shown in Fig. 4. $\times 1,000$.

Fig. 23. A young ascogenous hypha with entering nuclei. $\times 1,900$.

Fig. 24. An older ascogenous hypha with a single branch ; the nuclei lie irregularly. $\times 1,900$.

Fig. 25. An oogonium with ascogenous hyphae ; all nuclei in the latter and some in the former are undergoing simultaneous mitosis ; those in some of the hyphae are already in telophase. $\times 1,900$.

Fig. 26. Part of an oogonium showing a group of nuclei in anaphase. $\times 1,900$.

Fig. 27. A branch of an ascogenous hypha from an oogonium like those shown in Figs. 25 and 26 ; five nuclei are in simultaneous mitosis ; a slight lag from tip to base may be noted. $\times 1,900$.

Fig. 28. A group of ascogenous branches ; in one the daughter nuclei of the previous mitosis still lie side by side ; the tips of the hyphae have the appearance of active growth. $\times 1,900$.

Fig. 29. A branched filament at a stage similar to that shown in Fig. 28. $\times 1,900$.

Fig. 30. Simultaneous mitosis in a branch somewhat older than those in the preceding figures. $\times 1,900$.

Fig. 31. Anaphase in a similar hypha ; eighteen of the twenty-four chromosomes can be counted. $\times 2,600$.

Fig. 32. Telophase in a similar hypha ; the lag from tip to base is evident. $\times 1,900$.

Fig. 33. Very late telophase ; the daughter nuclei are reconstructed and have been pushed apart by the vacuoles lying between them ; one is isolated in the tip and another near the base. $\times 1,900$.

Figs. 34, 35. Progressively later stages. $\times 1,900$.

Fig. 36. A rather older hypha in which wall-formation is in progress ; the second wall from the base is shown in two foci so as to indicate the annular method of formation. $\times 1,900$.

Figs. 37, 38, 39. Older hyphae in which the binucleate cells are budding out to form croziers ; the uninucleate tip cell can be seen in each case. $\times 1,900$.

Fig. 40. A binucleate cell, the tip of which is just beginning to bend over in crozier formation. $\times 1,900$.

Fig. 41. Crozier formed by a binucleate cell ; the binucleate cell above it is beginning to grow out ; the branch ends in a uninucleate cell. $\times 1,900$.

PLATE XIV.

Figs. 42, 43. Stages in the development of a trinucleate crozier. $\times 1,900$.

Fig. 44. Prophase in the two nuclei of a crozier ; each shows twelve chromosomes. $\times 2,600$.

Fig. 45. Anaphase in one of the nuclei of a crozier, twelve chromosomes going to each pole. $\times 2,600$.

Figs. 46, 47, 48. Telophases in the crozier ; Fig. 47 is viewed from behind ; Fig. 48 shows the uninucleate tip-cell of the branch from which the crozier has arisen. $\times 1,900$.

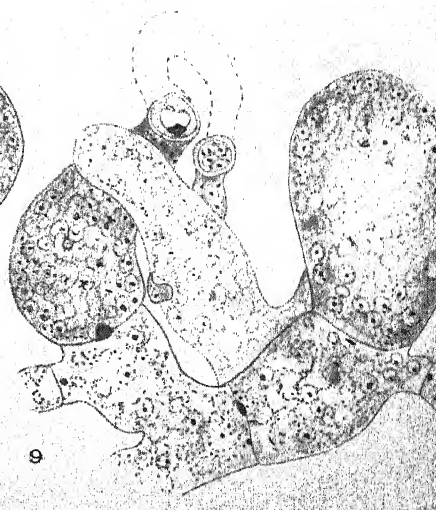
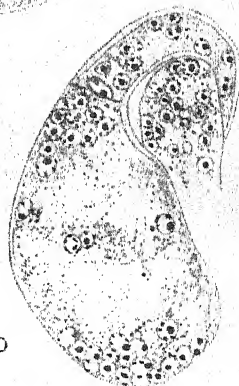
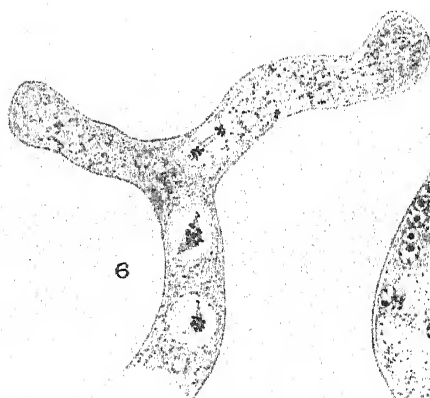
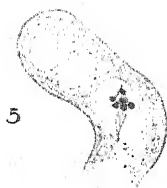
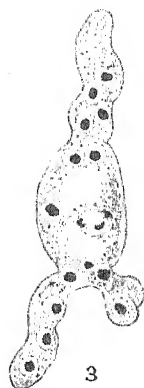
Fig. 49. An ascogenous branch ending in a uninucleate tip-cell and bearing two croziers in each of which mitosis is complete, and the uninucleate stalk and terminal cells have been formed. The branch can be traced through a somewhat broken, empty region, to the main ascogenous hypha, which still retains a group of nuclei and is attached to the oogonium. $\times 1,900$.

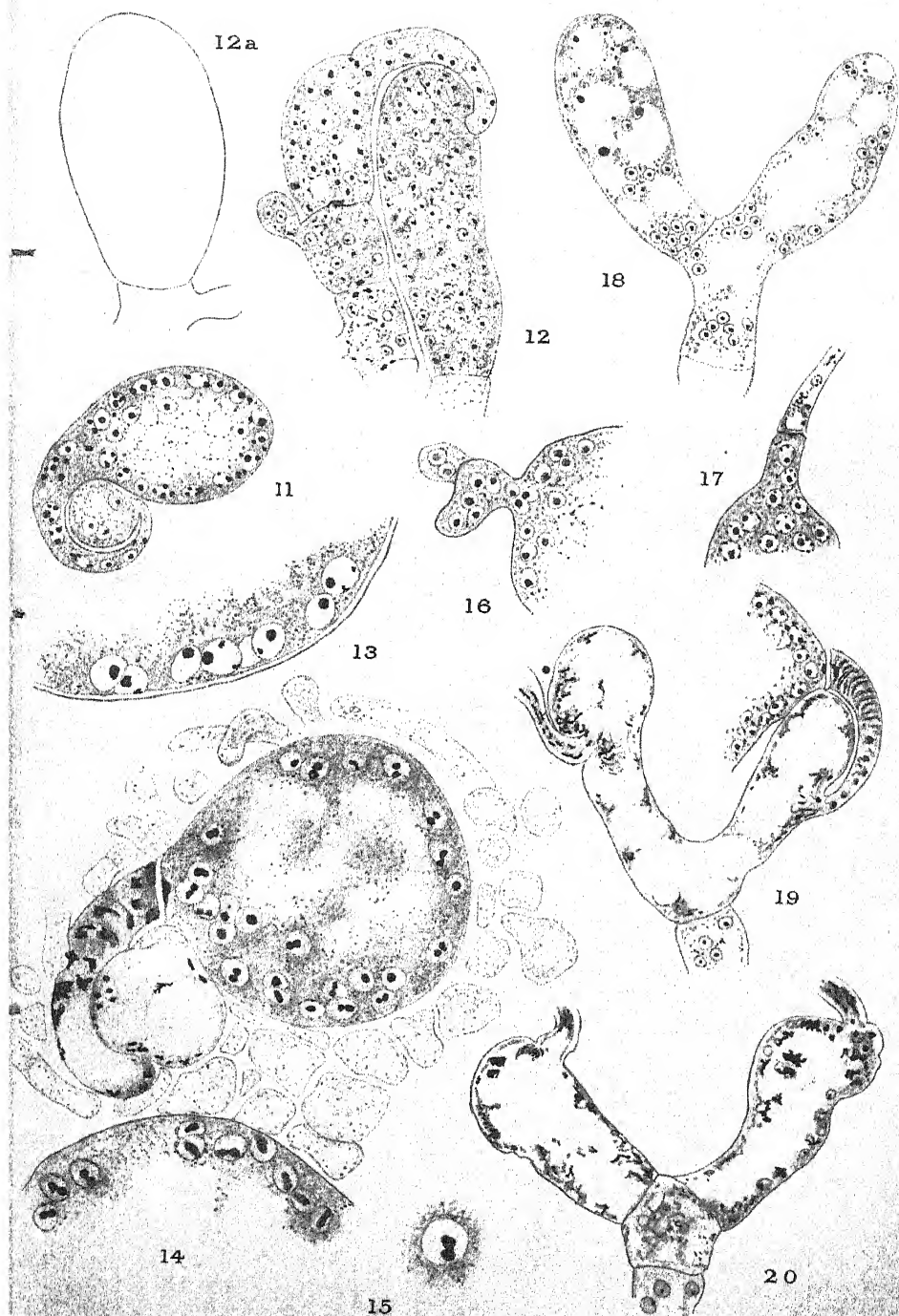
Fig. 50. A crozier showing fusion in the ascus cell. $\times 1900$.

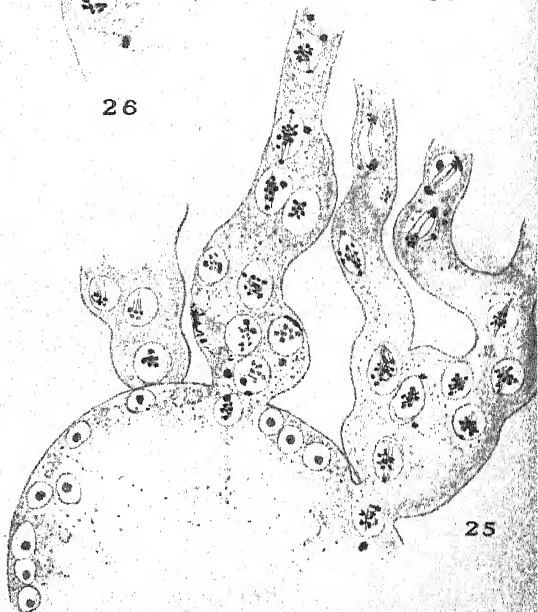
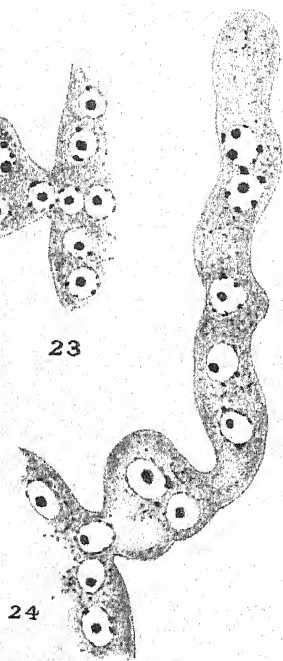
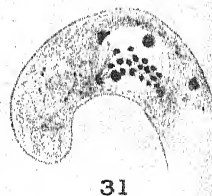
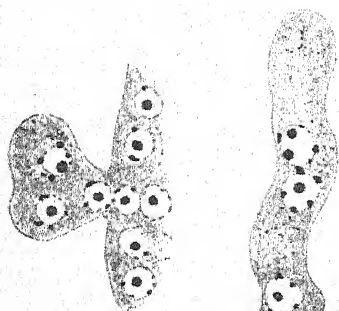
Fig. 51. The first meiotic telophase in the ascus, with twelve whole chromosomes going to each pole. $\times 2,600$.

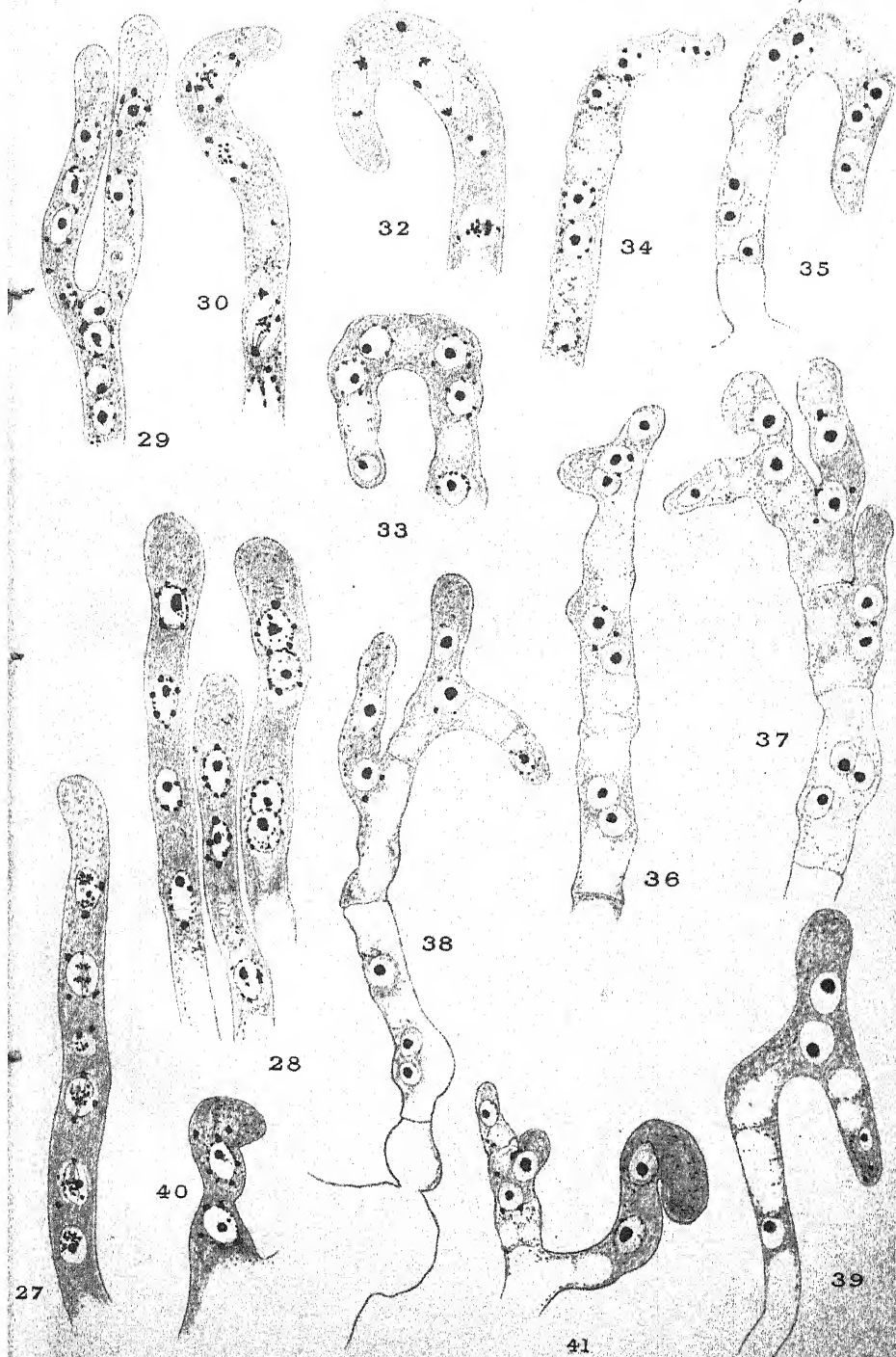
Fig. 52. Metaphase of the second division in the ascus, showing six chromosomes. $\times 2,600$.

Fig. 53. Third division in the ascus ; the lower nuclei are in late metaphase, the next shows an anaphase, and that nearest the apex an early telophase in which six chromosomes can be counted at the pole. $\times 2,600$.

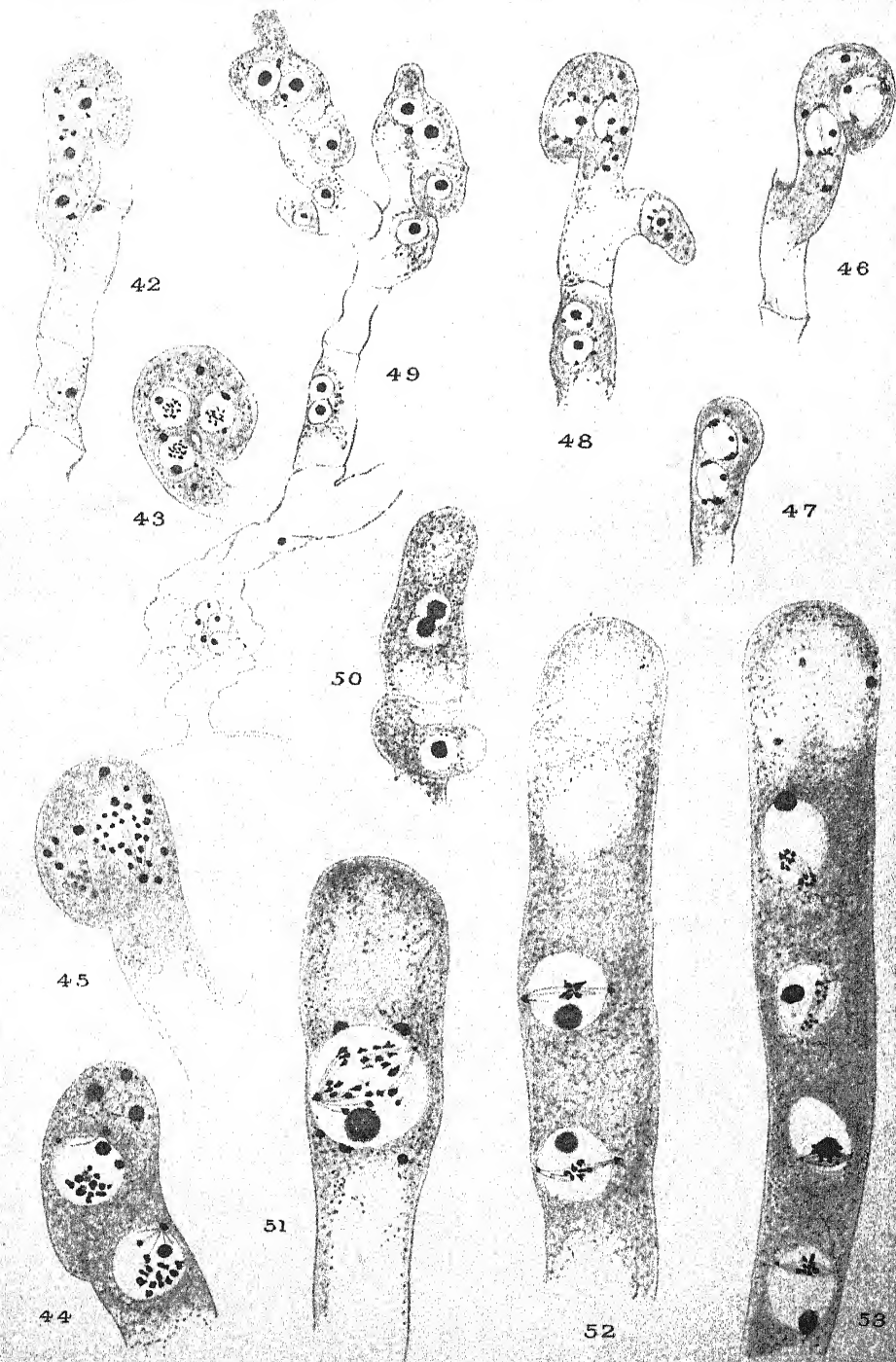














NOTES.

ON A SIMPLE ELECTRIC IMMERSION HEATER FOR CONSTANT TEMPERATURE BATHS.—With the advance of modern methods of physiological research necessitating an accurate control of experimental conditions, the need for a satisfactory constant temperature bath that can be easily fitted up is being repeatedly felt by workers in this field. It is true that electrically controlled baths are manufactured by a number of firms, but these, so far as the writer's experience goes, are either expensive or are not sufficiently constant in their operation for the majority of research purposes.

The use of carbon filament lamps as heaters is well known among physiologists, and these, working in conjunction with relays and thermo-regulators of the mercury or mercury-toluene type, have been repeatedly used and have given varying degrees of satisfaction.

The great drawback to the use of such lamps is that they give only a comparatively rough control of temperature. This is due to the large heat capacity of the lamp and its holder, which is often waterproofed in some way, as by paraffin wax, with the result that there is a relatively large lag in the temperature control. Accordingly, no matter how sensitive the thermo-regulator may be, its effect is limited by the lag in question. A similar drawback accompanies the use of many commercial electrical immersion heaters.

In the early stages of some researches that the writer has been engaged upon in collaboration with Professor W. Stiles, it was realized that water-baths maintaining a high degree of constancy of temperature would be required, and consequently it was found necessary to devise an electrical heating element that would not limit the action of a sensitive thermo-regulator.

A number of samples of 'Brightray' wire (a high-grade nickel-chromium alloy) of various gauges were obtained from Messrs. H. Wiggin & Co., of Wiggin Street, Birmingham. Spirals of this wire were formed by winding it with adjacent turns touching on a knitting needle of about 1.5 mm. in diameter. The spirals thus made were removed from the knitting needle, and No. 22 S.W.G. bare copper leads were silver-soldered to the ends of each spiral. The spirals with their copper leads were then pulled through lengths of 5 mm. bore glass tube that had been previously bent to form three sides of a rectangle (see Fig. 1). It was so arranged that the spiral only lay along the horizontal part of the tube, the vertical side tubes containing the copper leads.

It was found that when such a tube with its spiral was immersed in water the wire could be heated to a bright red heat by means of an electric current without cracking the glass.

After a considerable amount of experimenting it was finally decided that immersion heaters of this type, consuming about 100 watts, were the most generally useful for physiological purposes where temperatures from 20 to 40 degrees centigrade were required. It was found that a single heater sufficed for water-baths of 6 to 10 gallons capacity, while larger baths up to 40 gallons required two heaters.

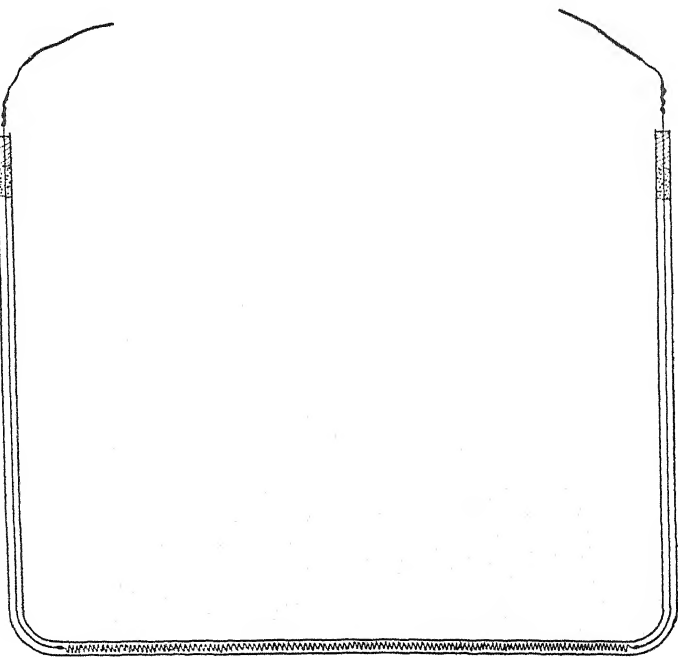


FIG. 1. For description see text.

The wire finally adopted as most suitable for the spirals, to work with a mains voltage of 220-230, was No. 40 S.W.G. 'Brightray', and the length used for each spiral was 12 feet, giving a current consumption of about 0.6 amperes. This length may be varied slightly if it is thought to be desirable, shorter or longer spirals giving higher or lower temperatures with correspondingly greater or smaller current consumption.

If the heaters are to be supplied with current from 110 volt mains, 12 feet of No. 37 S.W.G. wire should be used, when the consumption will be about one ampere.

The heaters were finished off by having the ends of the glass tube plugged with cotton wool and sealed with a wax such as sealing-wax. The ends of the copper leads were then cut short and soldered to ordinary 5 amp. flex and insulated with pieces of india-rubber tube threaded on the flex previous to soldering.

The heaters can be suspended in the bath by any suitable means, such as clamping to the sides with wooden strips. The containing glass tubes should be made of a suitable size to allow the horizontal member containing the spiral to hang about 4 inches from the bottom of the bath, and at least 1 inch from the side. Care

should be taken to avoid switching on the current when the heater is not immersed in water, otherwise it will crack.

In use the heaters were operated by means of the well-known mercury grid type of thermo-regulator, which may be fitted with either platinum, or nickel-chromium, mercury contacts surrounded by hydrogen or immersed in glycerine.

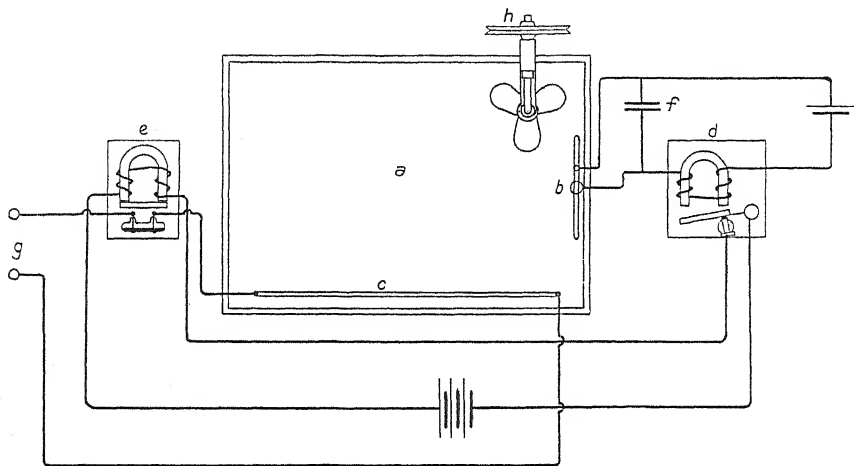


FIG. 2. Circuit diagram of constant temperature-bath: (a) water-bath; (b) thermo-regulator; (c) heater; (d) small relay; (e) mains relay; (f) 2-microfarad condenser; (g) terminals connecting to lighting mains; (h) stirrer.

Both the hydrogen and the glycerine types, with platinum wire contacts cut to fine points, were used by the writer and found satisfactory. The leads from the thermo-regulator were connected in series with a 2-volt accumulator and a relay made from an electric bell which required only a very small current to operate it. A 2-microfarad condenser was connected across the contacts of this relay, as shown in Fig. 2, in order to reduce sparking at the thermo-regulator contacts. This small relay was arranged to operate a larger one having a mercury break and which was capable of dealing with the voltage of the lighting mains (220 volts) and the current passed by the heaters.

The water in the bath was kept in a state of fairly rapid circulation by means of propeller-type stirrers having instead of gears, spiral flexible drives, to reduce noise. The stirrers were driven by an electric motor. Large baths were lagged with sheet asbestos while small ones were surrounded with cotton wool and placed in wooden boxes to prevent undue loss of heat.

With the method of heating control described it was easily found possible to maintain 6-gallon and 40-gallon baths at a temperature of 25°C. with a constancy of 0.05°C. using a thermo-regulator having a mercury grid formed from 140 cm. of 5 mm. bore glass tube. If a greater constancy is required, a regulator with a larger mercury grid or one of the mercury-toluene type should be used.

I am indebted to Dr. S. R. Carter, of the Chemical Department of the University of Birmingham, for a useful suggestion in connexion with the construction of

mercury-toluene thermo-regulators. Many workers will have experienced difficulty with these regulators because of the formation of a sulphide of mercury which often clogs the capillary round the contacts and prevents them from working. This difficulty is due to small amounts of sulphur compounds that are nearly always present in commercial toluene. These compounds can be removed by adding a little mercury to the toluene a few days before it is to be used for filling the thermo-regulator and occasionally shaking during this period.

N.B.—When using such electrically-heated baths it is advisable to have a thick copper wire with one end dipping into the water and the other end soldered or clipped to a water-pipe so as to form an earth connexion. In this way dangerous electric shocks are avoided in the event of a short circuit occurring.

W. LEACH.

ON THE PREPARATION OF CELLULOSE FILMS OF FOSSIL PLANTS.—In 1928,¹ Walton described a method of preparing sections of fossil plants contained in petrifications. The essence of this technique is to dissolve by means of a suitable acid a thin layer of the matrix in which the plant tissues are contained, and to replace the material dissolved by a cellulose solution, which, drying to a thin film, can be peeled off and carries with it a section of the plant tissues. In 1930 Barnes and the writer² described certain modifications of this technique, in particular the fixation of the film on a slide and the removal of the celluloid before mounting in balsam, so that thin sections of the plant tissues only were retained.

Experience of certain limitations of the method led to the investigation of the scientific basis of the use of cellulose compounds and solvents in the cellulose-lacquer industry, and experiments with both solvents and cellulose compounds on lines indicated by these principles have resulted in the elimination of the factors which operated against the production of a good film.

Films made with cellulose nitrate or cellulose acetate solutions are to a certain extent hard and brittle, and become progressively harder and more brittle with the passage of time, due to the gradual diminution of the amount of solvent remaining in the film. Moreover, films made from such solutions contract somewhat unequally on drying, causing 'puckering'.

Both of these disadvantages have been overcome by incorporating a small proportion of a 'plasticiser' into the solution. The plasticisers successfully used were castor oil, triacetin (glyceryl triacetate), and benzyl abietate (resin ether). The plasticiser used for any particular solution must of course be a solvent for the solid matter of which the film is composed. In addition to the elimination of brittleness of the film by the use of such plasticisers they render the film stable towards moisture.

¹ Walton, J. A. : A Method of Preparing Sections of Fossil Plants Contained in Coal-balls or in Other Types of Petrifications. *Nature*, cxxii. 571, 1928.

² Barnes, B., and Duerden, H. : On the Preparation of Celluloid Transfers from Rocks Containing Fossil Plants. *New Phytologist*, xxix. 74, 1930.

It was found that by using solutions made with low-boiling solvents, e. g. equal parts of absolute alcohol and ether, or acetone, a cloudiness sometimes appeared in the still liquid film, after the solution had been poured on to the rock surface. This was due to the too rapid evaporation of the low-boiling solvents cooling the air in the vicinity of the film below the dew-point of the atmosphere, with the result that water was introduced into the film and the precipitation of ingredients which are insoluble in water followed. Moreover, by the use of such low-boiling solvents the film increased in viscosity too rapidly and a bad 'flow' resulted.

Therefore, to control to some extent the rate of evaporation of the liquid medium of the solution, two-solvent solutions were used in which one of the solvents had a much higher boiling-point than the other; the solvent of higher boiling-point was employed in smaller proportions than the one of lower boiling-point.

In this way it was found possible to prevent the deposition of water from the atmosphere, to control the flow of the solution, and to prevent undue contraction of the film. In addition, the use of the higher-boiling solvents eliminates the risk of air-bubbles appearing in the film unless the rock is exceptionally porous. With all the coal-ball material used, air-bubbles never appeared in the film when solutions containing the higher-boiling solvents were used.

A certain yellowness and opacity of the films due to the use of waste celluloid as a source of cellulose, can be avoided by the use of cellulose acetate or of pyroxylin (gun-cotton), both of which give clear films.

Experiments have been made with a large number of combinations of low- and high-boiling solvents, plasticisers, and cellulose compounds. The following combinations were found to give excellent films.

A solution of pyroxylin in equal parts of alcohol and ether to which five per cent. of castor oil has been added, is good if the rock is not particularly porous and in consequence, air-bubbles are unlikely to appear in the film. This is a rapidly drying solution and gives a beautifully clear and elastic film.

A much slower drying solution, but one yielding a first-class film, consists of two parts of acetone to one part of amyl acetate in which two per cent. of triacetin has been incorporated; pyroxylin is used as a source of cellulose.

Excellent films have been obtained from a solution of cellulose acetate in four parts of acetone to one part of diacetone alcohol in which one per cent. each of benzyl abietate and triacetin has been incorporated, and from a solution of cellulose acetate in four parts of acetone to one part of benzyl alcohol and one per cent. each of benzyl abietate and triacetin.

In each of the above solutions sufficient of the cellulose compound was added to make the resulting solution about as viscous as pure glycerine.

In all cases the preliminary steps of grinding and etching the rock surface were, as previously described (2). Before pouring the solution on to the etched surface of the rock a mixture of the solvents was poured on.

In this way films may be obtained which are free from air-bubbles, and are perfectly smooth and level, thus rendering their subsequent mounting in balsam much easier; in addition, they possess a high degree of translucency, consequently more fine detail may be obtained from such preparations.

The methods described above have been successfully employed in the course of work in progress on coal-ball material from the Lower Mountain Mine seam of the Burnley coal-field, and on material of the fossil Osmundaceae from Cretaceous strata.

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NOTES ON THE EARLY STAGES IN THE DEVELOPMENT OF THE COTTON-FIBRE AND THE STRUCTURE OF THE BOLL AND SEED.—

Active work is in progress in different centres in India on the cottons, both indigenous and naturalized, but very little has, so far, been done from the view-point of cytology. It is the intention of the author to present in brief certain of the interesting facts which have come to his notice while working out the cytology of cottons.

Cotton-Fibre.

Conflicting opinions have, from time to time, been expressed with regard to the early stages in the development of the cotton-fibre, but nothing definite, has yet been established. Balls¹ says that *the density of lint on the boll is determined when the lint first originates by the protrusion of individual epidermal cells. There does not appear to be any further growth of epidermal cells into lint hairs after this first day, in spite of accepted statements to the contrary.* Turner² in a recent publication has criticised Balls, and his third view with regard to the early development of the cotton-fibre is fully supported by the cytological evidence obtained by the author and differs from that of Balls.

Observations have been made on cotton bolls of *Gossypium hirsutum*, *G. indicum*, and *G. neglectum*, up to forty-eight hours old. The specimens were fixed in acetic-alcohol according to Balls's method³, but the subsequent embedding method was that of Dawson⁴. Sections 10 μ thick were cut and stained in Heidenhain's iron-alum haematoxylin.

On the very first day that the flowers open certain of the epidermal cells of the ovule bulge out in the form of tiny balloons, each with a big nucleus and deep staining cytoplasm. These are sparsely distributed on the outer integument (Fig. 1). According to Balls⁵, these alone develop into cotton-fibres (including also the fuzz). It is a well-known fact that the fertilized ovule grows bigger as the boll ages, and so naturally the number of epidermal cells of the integument is also bound to increase. In bolls thirty-six hours after fertilization it has been observed that cotton-fibres in

¹ Balls, W. L.: Development and Properties of Raw Cotton, pp. 82-3, 1915.

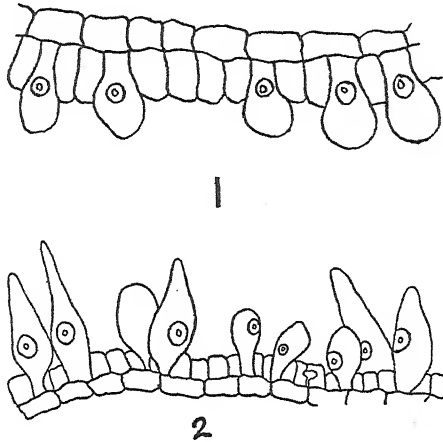
² Turner, A. J.: Ginning Percentage and Lint Index of Cotton in Relation to the Number of Cotton Fibres per Seed, &c. Indian Central Cotton Committee Technological Laboratory, Bull. No. 18, Technological Series, No. 13.

³ Balls, W. L.: loc. cit., pp. 175-6.

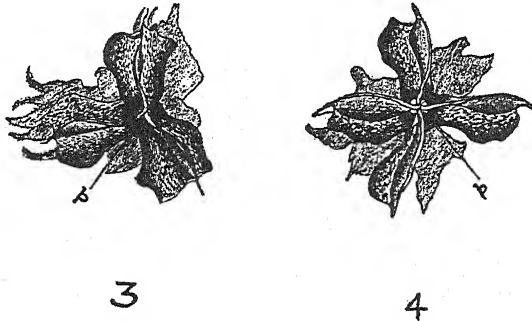
⁴ Dawson, W. J.: A New Method of Paraffin Infiltration. Ann. Bot., xxxvi, pp. 577-8, 1922.

⁵ Balls, W. L.: loc. cit., pp. 82-3.

different stages of development occur (Fig. 2) and in those forty-eight hours old almost all the epidermal cells have been found to be involved in the formation of the fibres.



FIGS. 1 and 2. Fig. 1. A portion of the longitudinal section of an ovule from the ovary of a freshly open flower. $\times 500$. Fig. 2. A portion of the longitudinal section of a fertilized ovule thirty-six hours later. $\times 300$.



FIGS. 3 and 4. Fig. 3. An adult dehiscid three-chambered boll showing the pits, *p*. $\times \frac{1}{2}$.
Fig. 4. An adult dehiscid four-chambered boll showing the pits, *p*. $\times \frac{1}{2}$.

Thus these observations are not in agreement with those of Balls, and conclusively prove that *the number of the fibres does increase during the life-history of the ovule.*

Boll

The interesting study of the boll has been neglected for a long time. Several systematists¹ have described the genus *Gossypium* but none has referred to the

¹ Bamber, C. J.: *Plants of the Panjab*, p. 90, 1916. Cook, T.: *The Flora of the Bombay Presidency*, i, pp. 115-19, 1903. Duthie, J. F.: *Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalayan Tracts*, pp. 94-97, 1903. Engler, A., and Prantl, K.: *Die Natürlichen Pflanzenfamilien*, III Teil, pp. 51-3, 1895. Nairne, A. K.: *The Flowering Plants of Western India*, p. 32, 1894. Kurz, S.: *Forest Flora of British Burma*, i, p. 129, 1877. Roxburgh, W.: *Flora Indica (Description of Indian Plants)*, pp. 519-22, 1874. Bentham, G., et Hooker, J. D.: *Genera Plantarum*, i, Part I, p. 209, 1862.

occurrence of such an important and constant character as the pits at the base of the boll-chamber inside. Hooker¹, has, however, described in the case of *G. Barbadense*, L., the ovary as pitted. But these pits, from the description, appear to refer to those present on its outer wall, and certainly they have no relation to the pits that are now described.

A study of adult as well as young bolls has been made. It has been found that in each chamber at the base there is one circular pit. This is a constant feature of all the Indian and Indian-American cottons thus far examined. These pits are, however, defined fairly late in the development of the boll and are cup-shaped (Figs. 3 and 4. Most of the fibres (usually of the basal region) are attached in these pits so that on tearing the seeds off the boll, a column of fibres (about $\frac{1}{10}$ in. diam.) remains firmly adherent to them; also quite a number of fibres remain attached to the sutures on the lower half of the boll. It is interesting to note that the attached-ends of the fibres microscopically examined in liquid paraffin are frequently branched like the haptera of benthos algae. Perhaps this has something to do with the nutrition of the cotton-fibre during its ontogeny. A microscopic study from this point of view is in progress.

Seed.

A recent paper by Barritt² is interesting in that he has described the structure of the seed-coat, and has drawn attention to the presence of '*palisade cells*' (one-layered) in both the outer and the inner integuments.

In some of the Indian *Gossypia* (e.g. *G. neglectum* and *G. indicum*) it has been found that the *palisade cells* are well developed and occur in one layer in the outer integument only, abutting directly on the epidermis of the second 'coat'. While in the inner integument (second 'coat'), cells in the homologous position never develop into the *palisade* layer and are reduced to a papery layer by the developing embryo. Exactly the same is the case with *G. hirsutum* with this difference, that the *palisade* layer of the outer integument encloses the developing embryo (along with the nucellus) half-way as if in a cup.

The *palisade* cells are not bottle-shaped as recently reported (by Barritt) for the genus *Gossypium*, but instead are shaped in longitudinal sections like an hour-glass or a tumbler.

The insertion and general morphology of the fibres, including even the branched ones, are in agreement with Barritt's observations.

I am thankful to Professor N. K. Tiwary of the Benares Hindu University, for his very kind provision of facilities for carrying out this work in his laboratory.

T. C. N. SINGH.

NORTH VIEW, SIMLA.

¹ Hooker, J. D.: The Flora of British India, i, pp. 346-7. 1875.

² Barritt, N. W.: The Structure of the Seed-coat in *Gossypium* and its Relation to the Growth and Nutrition of Root-hairs. Ann. Bot., xliii, pp. 483-9, 1929.

The Biology of Banana Wilt (Panama Disease).

III. An Examination of Sucker Infection through Root-bases.

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With Plate XV and twenty Figures in the Text.

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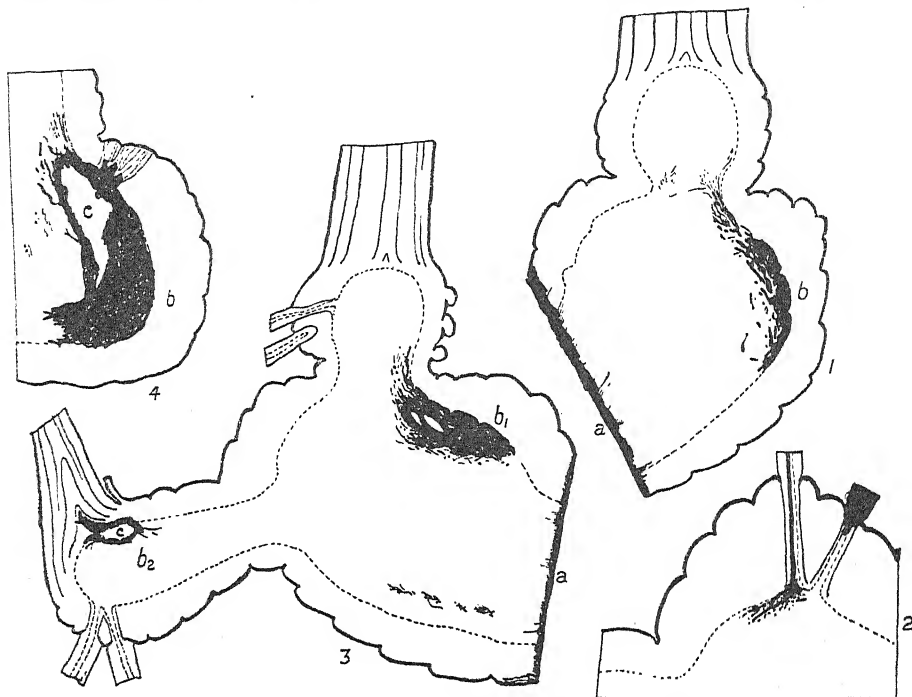
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I. INTRODUCTION.

IN the two preceding papers of this series (8, 9) some account was given of the attack of *Fusarium cubense* on the roots and suckers of the Gros Michel banana. While effective parasitism results in the production of a characteristic wilt (Panama Disease), it was found that uninjured roots growing under favourable conditions were not attacked by the fungus, and that healthy root or sucker tissue resisted deep penetration by the development of suberized cambiform tissue. The object of the present paper is to submit new observations on the process of sucker infection through diseased root-bases.

When grown in moist, well aerated soils, heavily inoculated with *F. cubense*, it was found from a detailed examination of at least 100 suckers, that effective penetration by the fungus never took place through the cut basal end, or through superficial wounds. Infection was invariably found

to be of lateral origin, associated with a diseased root-base or with a cavity produced by *Cosmopolites sordidus*. Text-fig. 1 shows a typical example of a sucker grown for eight months in infected soil under experimental conditions. Penetration at the cut basal end (*a*) is negligible, the



TEXT-FIGS. 1-4. Results of inoculation experiments at the end of eight months. 1. Sucker showing typical limited infection at cut basal end (*a*) and lateral infection at (*b*). The endodermis is indicated by the broken line, and infection by solid black. $\times \frac{1}{2}$. 2. The origin of a lateral sucker infection from a diseased root. The diseased strand passes through the root-base to the sucker-stele. The other root-base shows a typical limited infection. $\times \frac{1}{2}$. 3. Sucker and daughter sucker showing lateral infections at (*b*₁) and (*b*₂) associated with borer cavities. The basal end (*a*) again shows limited infection only. $\times \frac{1}{2}$. 4. Another section from the same sucker showing the large borer cavity (*c*) surrounded by diseased tissue. $\times \frac{1}{2}$.

important infection being of lateral origin at (*b*). Text-fig. 2, from the same experiment, shows the origin of such a lateral infection through a diseased root-base. Text-fig. 3 also taken at the end of eight months shows a lateral infection associated with a borer cavity (*b*₁). Here again penetration at the basal end is of the slightest nature. A second lateral infection, also associated with a borer cavity, is shown at *b*₂ in the daughter sucker.

In the production of banana wilt Brandes (1) has described two infection courts, namely, unwounded young roots and wounded areas of the rhizome. Some revision of views regarding infection courts, however, now seems necessary. Basal infection of the sucker is, of course, found where

the parent rhizome is infected, and where the diseased condition has been transmitted to the daughter sucker. It is possible that under certain acutely adverse soil conditions, basal infection may also take place, but so far examples have not come to the writer's notice. On the other hand it has been definitely ascertained that in soils of sufficiently open texture, basal infection does not take place. It is true, as Brandes observed, that the hyphae of *F. cubense* penetrate through the wounded tissue of the rhizome. The writer has shown, however, that this penetration is of limited extent, and is held in check by the formation of suberized cambiform barriers (9). Such barriers may be developed in any region of the sucker where conditions of moisture and oxygen supply permit of the rapid growth activities of living parenchymatous tissues.

When the basal end of the sucker is cut through, the numerous vessels exposed and injured should theoretically provide easy channels for the rapid ingress of hyphae. This, however, does not happen, and in actual practice vascular penetration at the basal end remains inextensive, while discoloration of bundles seldom exceeds 1 to 1½ cm. Some observations on this subject are submitted in the next section.

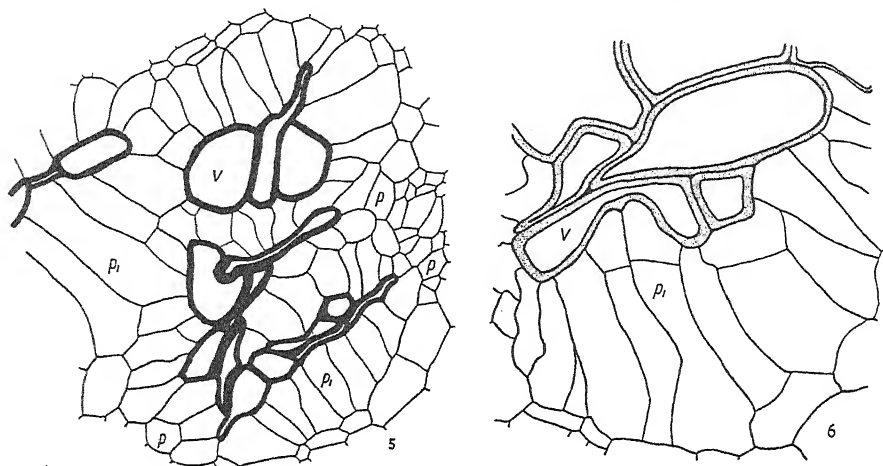
It was further demonstrated that at another point where superficial infection might take place, namely, the old leaf-bases, similar protective suberized cambiform layers were also present. Thus, under the experimental conditions described, the question of sucker infection has been narrowed down to a study of lateral penetration. It has been seen that this may take place through the agency of boring organisms or through diseased root-bases. In the present paper detailed observations on the latter aspect of sucker penetration are set forth.

For purposes of comparison a brief account of the limited vascular penetration at the cut basal end will first of all be given.

II. VASCULAR PENETRATION AT BASAL END OF SUCKER.

The material used for these observations was obtained as follows. Suckers of uniform size were trimmed, cut clean across at the basal end, and planted in soil heavily inoculated with *F. cubense*. Various soils were used, but in all cases a quantity of sand was added and intimately mixed so as to give a texture permitting of adequate aeration. Suckers were examined at intervals from four to six days after planting to eight months. In all at least one hundred suckers have been examined in detail, and as already described and illustrated by Text-figs. 1-4, no significant basal penetration was found. Penetration of the ground parenchyma is held in check by the formation of well-defined suberized cambiform barriers, and it now remains to consider what happens in the vascular strands, many of which are cut through by the separation of the sucker from the parent rhizome.

In suckers eight months old it was found that the amount of vascular discoloration did not as a rule extend inwards from the cut surface more than 1.5 to 2 cm. This condition, which was found to be established at



TEXT-FIGS. 5 and 6. 5. Collapsed vessels (*v*) at the basal cut end of sucker, resulting from the considerable distension of parenchymatous cells (*p*₁). The normal size of these cells is seen at (*p*₁). $\times 140$. 6. Collapse of vessels (*v*). The enlarged parenchymatous cells have commenced to divide (*p*₁). $\times 250$.

a very early stage, is mainly a result of wounding, as it is also found in suckers which have not been inoculated with *F. cubense*.

Longitudinal sections through this region reveal brown, red, or yellow vessels and tracheides whose structure is difficult to follow in detail. These vessels may contain hyphae of *F. cubense* or they may not. Owing to the presence of wound gum the hyphae are often difficult to see, and the use of various stains has not been found very helpful in this connexion. On the whole, sections from fresh material, mounted directly in water, or lightly stained with Kleinenberg's or Delafield's haematoxylin, and mounted in water or dilute glycerine were most useful. In the outermost portions of such longitudinal sections, hyphae were frequently found embedded in the reddish-brown gum of the vessels. That such hyphal growth is quickly inhibited is shown by the fact that deeper penetration was not found, even at the end of eight months. It was frequently found, however, that sections kept in water over-night showed a renewal of hyphal growth.

Transverse sections (i. e., parallel to the basal cut surface) proved more useful. Several series were prepared proceeding from the outer exposed surface inwards till discoloration of vessels disappeared. It was found that the vascular tissues had undergone important modifications. As the result of pressure exerted by the enlarging conjunctive parenchyma cells (Text-fig. 5), the red, brown, or yellowish wood vessels had undergone

a more or less complete collapse. It has already been seen (9) that both the cortical and stelar parenchyma of banana suckers and roots respond to wounding or invasion of adjacent tissue by a rapid enlargement, accompanied by cell-division and the deposition of suberin in the cell-walls. While the pressure exerted by the enlarging parenchyma must be very considerable, the appearance of the vessels suggests that the lignified walls have been softened to some extent, possibly by enzymes secreted by the invading organism (Text-fig. 6). Such collapsed vessels were found to be the rule in the tissue under discussion. The enlarged conjunctive parenchyma, in contrast to the vessels and tracheides, was invariably translucent and free from either discolorations or hyphae, denoting the presence of impervious walls by which the hyphae and their toxic solutions were being kept within the lumina of the vessels. Tyloses were not observed. The collapsed state of the vessels, together with the plugging and growth-inhibiting action of wound gum, are responsible, therefore, for the limited fungal penetration in this region of the sucker.

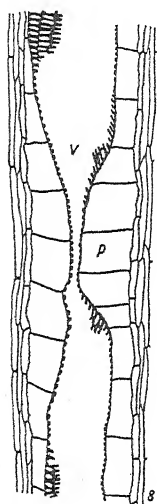
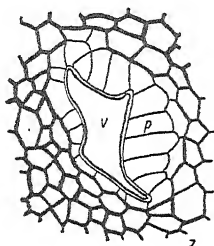
III. PENETRATION THROUGH ROOT-BASES.

In practice, before being planted, suckers are usually trimmed by cutting off roots close to the sucker. Each cut root thus presents an open surface which may readily be attacked by fungi, while on *a priori* grounds the vessels exposed appear to offer an easy route for the passage of hyphae from the soil into the stele of the sucker. When such a trimmed sucker, with twenty or more root-bases exposed, is planted in soil heavily inoculated with *F. cubense*, it might be expected that dissection after a reasonable lapse of time would reveal a considerable number of infections at the junctions of root-steles and sucker-stele. An extensive examination of root-bases, exposed in highly infected soils for periods varying from fourteen days to eight and ten months, however, did not reveal this plurality of infections. Where they were found at a sufficiently early stage they could generally be traced to one or two infected root-bases. The occurrence of only a few significant penetrations suggests immediately the presence of some disease-controlling mechanism.

As indicated in Text-fig. 1, the sucker consists of a large inner stele and narrow outer cortex which varies from 1 to 2 or more cm. in thickness. Roots have their origin at the periphery of the stele and pass out through the cortex to the soil. When they are trimmed off close to the sucker, parasitic hyphae in contact have therefore only to penetrate a short distance to reach the sucker-stele, and as already stated, this might be expected to take place quickly by way of the continuous system of wide wood vessels.

The nature and extent of root-base infections were examined from

transverse and longitudinal sections. After trying out a variety of staining methods it was found that observations could most readily be made on



TEXT-FIGS. 7 and 8.
7. Transverse section showing collapse of vessel (*v.*) in root-base as the result of expansion of surrounding parenchyma (*p.*). $\times 140$. 8. Longitudinal section. $\times 80$.

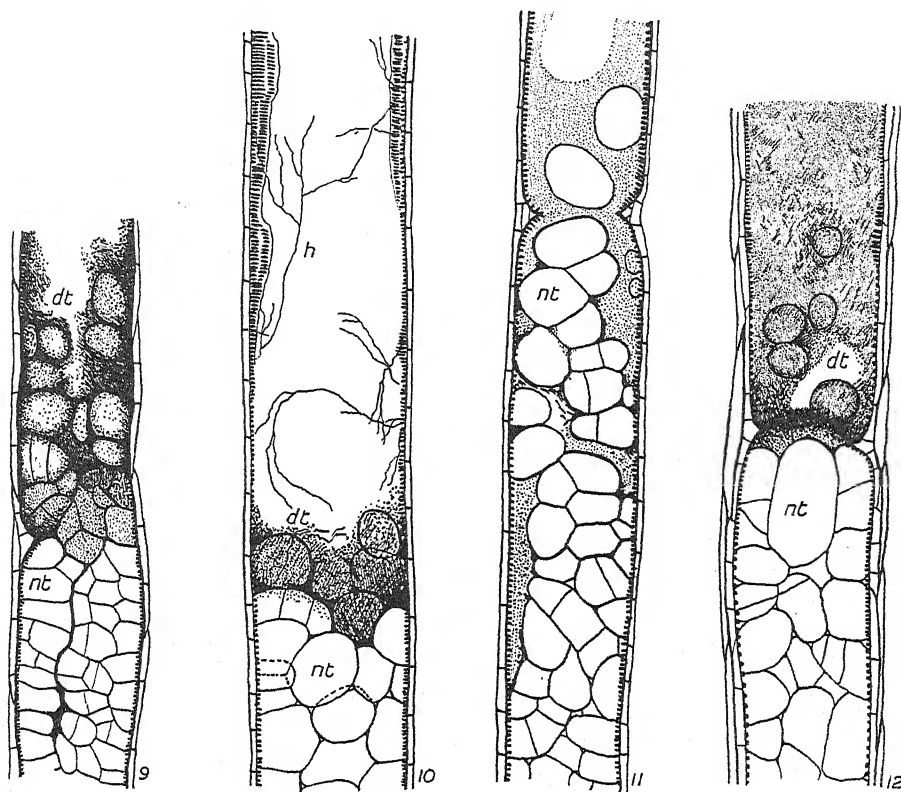
thin sections from fresh material mounted in water, or lightly stained with haematoxylin. The majority of root-bases showed a slight penetration only, the dark decayed outer region being relatively inextensive and frequently sharply delimited. An instance of this is seen in the right-hand root-base in Text-fig. 2. In some root-bases a few of the vessels were infected almost to the sucker-stele. The latter may be reached by hyphal penetration through (*a*) the cortical cells of the root-base, (*b*) the phloem and parenchyma cells of the root-stele, or (*c*) the wood vessels and tracheides. Penetration through the root cortex is usually held in check by the formation of suberized cambiform barriers; these traverse the cortex, frequently slanting outwards at an acute angle. Penetration through the living cellulose tissues of the stele is checked by similar protective mechanisms. The most significant paths of entry, namely, the vessels and tracheides remain to be considered. In Part II (9) a brief description was given of vessel collapse in root-bases. This results from pressure exerted by the adjacent conjunctive parenchyma cells which, by enlarging in response to fungal secretions or to other wound stimuli, crush the vessels and close them to invading hyphae (Text-figs. 7 and 8).

The most important mechanism for preventing fungal penetration through the vessels consists in the rapid development of tyloses.

IV. TYLOSES.

In Text-figs. 9–12 some typical modified vessels are illustrated, and it will be seen that where the whole cavity is occupied by tyloses, the inward progress of decay is definitely checked. In marked contrast to the outer diseased regions of the vessels, which are stained a deep yellow, red, or brown colour, the compact mass of living tyloses has a colourless transparent appearance. In the outer segments of the vessels, where the tyloses are not fully expanded, fungal hyphae may be observed. Occasionally vessels were observed containing many hyphae, but as a rule the fungus was not present in any great abundance. From such material all stages in the growth of tyloses may be studied. Where development is tardy the individual tylosis may be killed, and by careful focussing its disintegrating

nucleus and cytoplasm, of a characteristic brown colour, may readily be observed. Such tyloses acquire the yellow or brown colour of the gum in which they are immersed. Text-figs. 9, 10, and 12 show typical examples



TEXT-FIGS. 9-12. Infected vessels with tyloses; diseased tyloses (*dt.*); normal tyloses (*nt.*); hyphae (*h.*). $\times 250$. For description see text.

of partially developed tyloses which have been killed, and have acquired the general colour of the decayed portions of the vessels. In contrast to necrosed tyloses, healthy ones have a translucent appearance with the protoplasmic arrangements of normal parenchymatous cells. The outer walls adjacent to the infected regions of the vessels are impregnated with suberin.

As in the case of other invaded parenchymatous tissue, the outermost tyloses are sometimes killed by toxic solutions. This is probably to be attributed to the absence of a suberin deposit in their walls in the early stages of development.

Tyloses thus show the same general reactions as the parenchyma cells from which they are formed. The detailed illustration in Pl. XV gives some idea of the relationship between the advance of hyphae through the vessels

and the development of tyloses. The section, taken through the outermost portion of a root-base, was obtained from a sucker which had been kept for fifty days in a humid aerated desiccator after inoculation with *F. cubense*. A considerable number of small tyloses at various stages of expansion were present. Evidently these had been killed at an early stage, and as a result they had the characteristic appearance and colour of decaying tissue, the cell-contents consisting of granular cytoplasm coagulated in masses round the nucleus. The death of these young tyloses is most probably to be attributed to the action of toxic secretions, in the absence of suberin in the expanding walls. In some preparations the walls of tyloses were observed to be exceedingly thin and fragile, as if they had been subjected to the solvent action of fungal secretions. The vessel cavity in Pl. XV was occupied by a yellowish-brown gum in which both hyphae and tyloses were embedded. It has already been indicated that wound gum exercises an inhibiting action on the growth of *F. cubense*. This necrosed condition only prevailed in the outermost segments of the vessel. Further in the vessel cavity was completely blocked by tyloses.

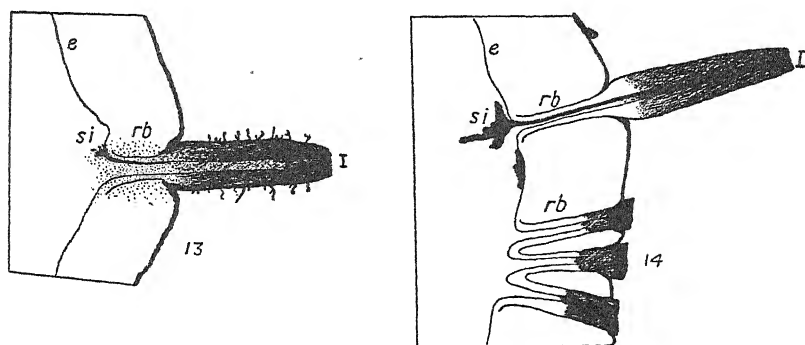
Discussion. Penetration by hyphae through the vessels of the root-base is, in the majority of cases, prevented by vessel collapse, by the development of massed tyloses with suberized outer walls, and by the secretion of wound gum. Where these protective devices come into play sucker infection by way of root-bases does not take place. For the formation of tyloses and the deposition of suberin, however, certain external conditions are required. These are discussed in a later section.

The foregoing observations have been concerned with the root-bases of suckers grown in moist, well-aerated soils, i.e. the ideal conditions for the activity of living tissues and for the formation of tyloses and deposition of suberin. On the other hand, soil conditions are known which permit of a very complete destruction of roots close to the sucker. The diffusion of toxic secretions from a highly decayed root, by inhibiting important vital activities in the root-base, probably constitutes an important stage in the infection of the sucker. On this subject Brandes has very rightly pointed to the significance of those roots which are highly decayed close to the sucker. 'Blackened roots close to the bulb and extending into diseased portions of the stele are frequently found, and this condition has been proved to be due to the banana-wilt organism.'

V. INITIAL STAGES OF SUCKER INFECTION.

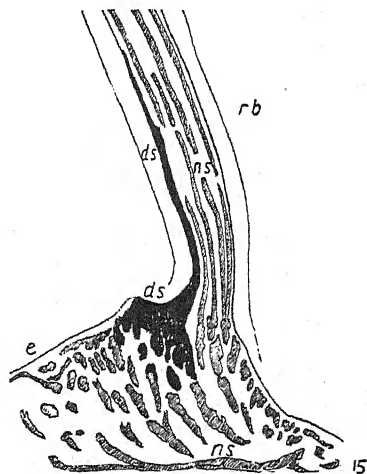
To obtain information on the initial stages of sucker infection some experiments were carried out along the following lines. Suckers growing in compost were carefully removed after sufficient time had elapsed to permit of root formation. After careful washing the roots were trimmed off close to the sucker, so that only 1.5 to 2 cm. of root remained. Their

cut ends were then inoculated with *F. cubense* (on an agar medium), and the suckers placed in closed moist desiccators for twenty-four days. During



TEXT-FIGS. 13 and 14. Initial stages of sucker infection. Roots cut and inoculated at (1) have become blackened and decayed. From the mass infection a red diseased vascular strand has passed through the root-base (*r.b.*) and infected the sucker just inside the endodermis (*e.*). Sucker infection (*s.i.*). Text-fig. 14 also shows the normal limited infection of root-bases. $\times 1\frac{1}{2}$.

this period the inoculated roots showed a slow die-back with blackening of tissue. Uninoculated control roots remained white. Different rates of decay were observed in different roots. In some the progress of the fungus was checked by gum secretion, vessel-collapse, and tyloses in the root-base, so that infection of the sucker stele did not take place. In others, however, definite infection of the sucker-stele was observed. Typical observations are illustrated in Text-figs. 13, 14, and 15. Longitudinal sections, through badly diseased roots, showed both root-stele and cortex to be of an inky bluish-black colour, with a water-soaked appearance. From this mass infection a characteristic red vascular strand passed inwards through the root-base to the sucker-stele, where the fungus commenced to spread to other vascular strands (Text-figs. 13 and 14). The course of infection is shown in greater detail in Text-fig. 15, where the spread to the vascular strands in the sucker was indicated by the production of characteristic red and yellow colours. Such sections show practically the earliest stages in sucker infection. Text-fig. 14 also shows some old root-bases with the typical limited



TEXT-FIG. 15. Detailed study of infected root-base (*r.b.*) as seen in longitudinal section. The diseased vascular strand (*ds.*) has carried the infection into the sucker-stele; (*e.*) endodermis; (*ns.*) normal vascular strands. $\times 7$.

amount of decay. From the examination of such material it was found that the disease was frequently transmitted to the sucker-stele along a single vascular strand, peripheral strands being most commonly affected. The red colouration was localized in the vessels and adjacent thick-walled fibres. Some longitudinal sections showed the vessels to be full of hyphae, with chlamydospores and non-septate and one-septate conidia. Occasional vessels showed tyloses, and others appeared to be free from hyphae. The outer black decayed portion of the root, all of whose tissues were penetrated by hyphae, may be regarded as a region of mass infection, from which toxic solutions diffuse inwards through the root-base to the sucker. This was indicated by the slightly discoloured and water-soaked appearance of the whole root-base seen *en masse*. It was observed that those roots which showed the greatest amount of decay had received a slight wrench at the point of emergence on removal from the soil. This effective parasitism is probably referable to several factors, including vitiation of the air in the closed desiccators, shock sustained by removal from the soil, cutting, and change of environment. In many of the inoculated roots the infection remained localized; microscopic examination at some distance from the outer decayed tissue revealed vessel collapse, gumming and tyloses, indicating the efficacy of these mechanisms in preventing deeper penetration.

VI. FURTHER OBSERVATIONS ON ROOT INFECTION.

It has been seen that infection of the sucker is consequent on the successful passage of hyphae through the root-bases. In the field, sucker infections are associated either with borer cavities or with those roots which are in an advanced state of decay close to the point of emergence. In Part I of this work (8) it was demonstrated that partial drying out of open soils, in the presence of the pathogen, resulted in the production of varying amounts of root disease from superficial blemishing to complete rotting. These observations have now been extended along experimental lines.

Experiments were carried out as follows. Eighteen containers (9.5 × 9.5 × 13 in. deep) were filled with potting compost, and planted with small uniform Gros Michel suckers. The latter were deeply set in the soil so as to eliminate drought effects in the uppermost roots. At the time of planting a culture solution, heavily inoculated with several strains of *F. cubense*, was poured freely over the sucker and into the surrounding soil. The containers were then uniformly watered each day.

At the end of sixteen days three suckers were carefully removed for examination. In all, some seventeen main roots were present, and close inspection showed these to be white, turgid, abundantly supplied with root-hairs and rootlets, and quite free from any discoloration or trace of disease. This bears out the writer's previous observation that under favourable soil conditions root infection by *F. cubense* does not take place.

Some of the roots were immediately cut off, placed in water, and taken into the laboratory. Longitudinal sections, prepared from material taken from 1 to 2 cm. behind the root-apex, were then immersed in a series of solutions of NaCl in water. It was found that a 1 per cent. solution brought about a considerable amount of plasmolysis from which, however, there was a rapid recovery on irrigation with water. Higher concentrations, e.g. 2 per cent., brought about a considerably more marked plasmolysis.

Six containers were accordingly watered with 2,000 c.c. of 1 per cent. salt solution, and six more with 2,000 c.c. of 2 per cent. salt solution, the latter to allow for buffering action by the soil. The solutions were allowed to act for one day, the pots being watered the following morning and on subsequent days as usual. The active salt solution would quickly be displaced by this process. The suckers were removed for examination at the end of twenty-one days. Three untreated controls, with a total of thirty-seven days' exposure to *F. cubense*, showed no root infection. The six containers which had been watered with 1 per cent. salt solution likewise showed no root disease. On the other hand, the roots in the six containers treated with the 2 per cent. solution showed a uniform type of disease effect.

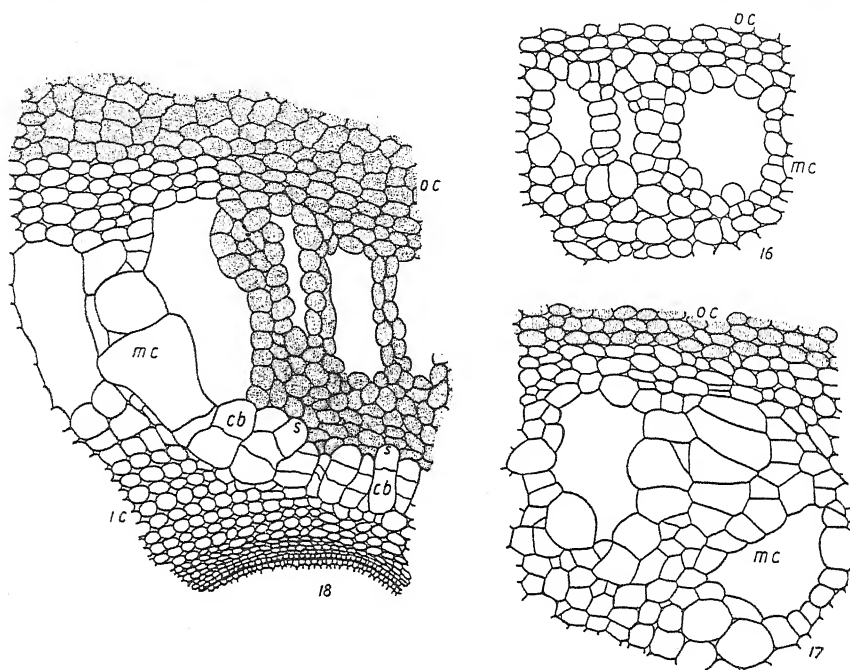
The roots could be separated into two categories :

- (1) Older roots which were diseased.
- (2) Younger roots which were free from disease.

The latter had developed during the last three weeks, and had not been subjected to plasmolysis.

Detailed observations. The affected roots showed all stages of infection, from superficial blemishing to complete rotting.¹ Badly affected roots had a limp, bluish-black appearance. Reddish or yellow vascular discolorations were not in evidence at this stage. Some short roots suffered a serious die-back, extending from the apex to the root-base, but in many others only the tissue in the vicinity of the apex was affected. In cases where the physiological shock had been relatively slight only the outer cortex was diseased, and the deeper penetration of invading organisms was held in check by the presence of typical suberized cambiform barriers similar to those already described in Part I (8). In such infections various organisms may, of course, be involved as unsterilized soil was used. On the other hand, root disease in the field is also liable to be influenced by a plurality of organisms. The dead outer cortex of affected roots was freely invaded by hyphae, but inside the suberized cambiform formations the tissue was normal and free from hyphae. Cells of the middle cortex where the tissue is less compact, frequently showed great enlargement (Text-figs. 16, 17, 18).

The only other type of disease effect observed was that which occurred where roots had rested against the metal wall of the container. Such roots (mainly root-tips) had acquired a rusty appearance. Microscopic examina-



TEXT-FIGS. 16, 17, 18. Transverse sections of roots in which infection has been promoted by plasmolysis. 16. Uniform parenchymatous tissue of unaffected middle cortex. Outer cortex (o.c.); middle cortex (m.c.). 17. Shows diseased outer cortex (o.c.), stippled, and enlarged cells of middle cortex (m.c.). 18. A more advanced infection; diseased tissue is stippled; the cells of the middle cortex are greatly distended, and a suberized cambiform barrier (c.b.) has been set up in the inner cortex (i.c.); suberized walls (s.). $\times 90$.

tion revealed the presence of decayed tissue under the superficial rust, with typical suberized cambiform barriers further in. The latter, therefore, constitute the general reaction to any type of wounding or physiological disturbance.

The foregoing observations show that, under the soil conditions of the experiment, a solution of NaCl whose concentration lies between 1 per cent. and 2 per cent., is capable of producing a physiological disturbance in the root-tissue of such a nature as to permit of fungal attack.

In order to eliminate any special action or toxicity on the part of common salt it was decided to substitute the isosmotic equivalent of cane sugar, using Pfeffer's figures for this purpose (4). It was found that segments of roots 3 cm. long when placed for twenty minutes in 20 per cent. solution of cane sugar became soft and limp; those placed in 15 per cent. were also soft and limp, but to a less marked degree; those in 10 per

cent. solution appeared to be little affected, while those in 5 per cent. did not appear to be affected at all. When replaced in water the affected roots regained turgidity. While complete recovery apparently takes place it is probable that a severe plasmolysis induces some degree of injury in the protoplasm of the more superficial cells.

The experiment on root infection was repeated as follows. After fifteen days' growth in inoculated compost four pots were treated with 2,000 c.c. of 1.5 per cent. NaCl solution, four with 2,000 c.c. of 13.5 per cent. cane-sugar solution, and four with 2,000 c.c. of 18 per cent. of cane-sugar solution (the isosmotic equivalents of 1.5 per cent. and 2 per cent. NaCl solutions respectively). The suckers were removed at the end of fifteen days, and their roots examined for disease. The roots in the first two series, i. e. 1.5 per cent. NaCl and 13.5 per cent. cane sugar were found to be entirely healthy, while careful examination of the suckers did not reveal any internal disturbance.

On the other hand, the containers which had been treated with 18 per cent. cane-sugar solution (isosmotic with 2 per cent. NaCl solution) showed typical decayed roots resembling those found after treatment with 2 per cent. salt solution. The three control pots which had been uniformly watered throughout were free from disease. As before, not all the roots in the pots treated with 18 per cent. cane sugar were diseased, since new roots appeared after plasmolysis had been carried out. A numerical consideration of the amount of root disease yielded the following figures :

Control No. 1	.	.	.	39	roots	.	.	all healthy.
Control No. 2	.	.	.	42	roots	.	.	all healthy.
Control No. 3	.	.	.	30	roots	.	.	all healthy.

The suckers treated with 1.5 per cent. NaCl and with 13.5 per cent. cane sugar gave similar figures.

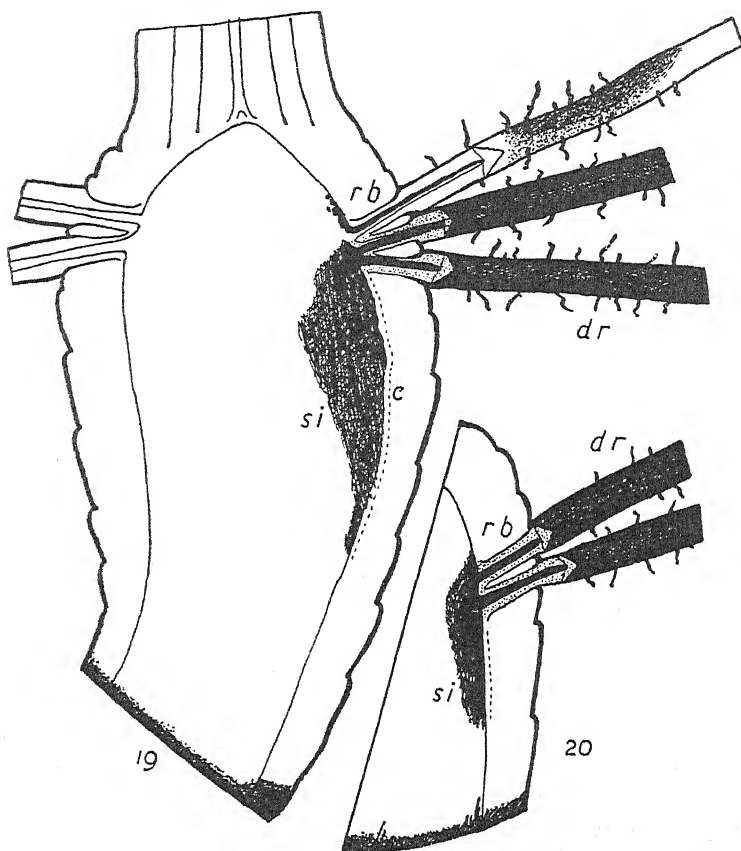
18 per cent. Cane Sugar.

No. 1	22 healthy ; 9 decayed to root-base.
No. 2	10 healthy ; 3 decayed to root-base.
No. 3	5 healthy ; 8 decayed to root-base.
No. 4	25 healthy ; 7 decayed to root-base.

The characteristic damage sustained by some of the first-formed roots in the vicinity of the apex is not included in the above summary, but as already indicated root disease in proximity to the sucker is of greatest importance.

Roots frequently occur in groups of two or three. In the vicinity of those which had become diseased to the base the sucker was carefully cut in longitudinal radial section so that the extent of internal infection might be examined. Typical sections are illustrated in Text-figs. 19 and 20. The

sucker-stele, in proximity to the diseased roots showed tissue which had been affected *en masse* and had acquired a water-soaked blue-black appearance. As the illustrations show, the diseased regions of the sucker were in direct



TEXT-FIGS. 19 and 20. Sucker infections (*s.i.*) which have resulted from plasmolysis and infection of roots. Diseased roots (*d.r.*); root-bases (*r.b.*); cambiform reaction (*c.*). For description see text. $\times 2/3$.

contact with the decayed roots, the infections having definitely proceeded inwards by way of the root-bases. In some of the latter the characteristic red colour was present, e.g., top root Text-fig. 19, but in the majority the tissue was of a bluish-black colour, like that of the affected sucker-tissue. On the periphery of the stele adjacent to the diseased tissue a zone of cambial activity could readily be seen with the naked eye. When a mycological examination of the discoloured sucker-tissue was made *F. cubense* and bacteria were isolated. No other fungus was obtained, while *F. cubense* was isolated many times from infected tissue of this kind. At this stage no red vascular discolorations in the sucker were observed. Similar sucker infections were found in conjunction with all the groups of badly

diseased roots. Some suckers thus showed three or four such infections. In contrast to these, unaffected root-bases had the appearance shown on the left-hand side of Text-fig. 19. No significant penetration at the cut basal end was observed in any of the suckers.

An attempt was next made to determine how long the plasmolysing solution had to remain in contact with roots in order to promote infection. The experiment was carried out as before, the plasmolysing solution (2,000 c.c. of 2 per cent. salt solution) being leached out after remaining in the soil for (a) half an hour, (b) one hour, and (c) two hours. On examination after twenty days it was found that varying amounts of root infection had taken place. In (a) this was exceedingly limited, the whole infection consisting of a slight blackening at root-tips. In (b) and (c), which showed similar degrees of infection, the amount of root decay was much more extensive, main roots being rotted from their apices backward over a length of 4 to 5 cm.

VII. THE INFLUENCE OF EXTERNAL FACTORS ON THE DEVELOPMENT OF PROTECTIVE TISSUES.

The reactions of parenchymatous tissues to wounding have been clearly discussed by Priestley and Swingle (6), while the causal factors operative in the formation of cork have been considered at some length by the first author (5). Their observations have an important application to the present work. They point out that when a parenchymatous tissue is cut through, the disorganization of superficial tissues will be followed by death and decay unless changes in the walls beneath the cut surface, such as to prevent the inward migration of micro-organisms, take place more quickly than the growth of the latter. 'Such changes in parenchymatous tissues seem usually to be brought about by the deposition, upon the carbohydrates in the wall, of a film made up of fatty substances which rapidly oxidize and dry, in the same way that similar films of unsaturated vegetable fatty substances, when exposed to the air, set to form compounds of a varnish-like consistency. Such films resist digestion and direct physical penetration by all micro-organisms if formed in time' (6).

In the Gros Michel, it has been seen that the end product of such changes, namely suberin, completely resists penetration by the hyphae of *F. cubense*. With regard to the present pathological investigation it is important to consider what external conditions are operative in the formation of suberin. Priestley and Swingle (6) have stated the position as follows. 'If the sap supply at the cut surface is adequate to give a continuous film of liquid, then as the fatty substances "cream" to the air-water surface the rapidity and effectiveness of suberin formation is dependent upon various factors. In particular, the free access of oxygen is essential. Thus, if the

cut surface is kept swimming in water, the fatty substances leach away and oxygen does not reach them while on the walls, and no suberin deposit is formed. This condition is fatal to successful suberization, and no single factor is so likely as an excess of water to produce decay at the cut surface.' Küster (3) also has indicated that oxygen supply is essential for effective suberization of injured surfaces. Other factors may also be involved. For example, in discussing wound reactions and callous formation, Küster states that the amount of callous formed bears a direct relation to the nutritional status of the organ itself. The same may well be applicable to suberin formation, for it has been seen that this process depends directly on the quantity and quality of sap at the wounded surface. According to Herklots (2) suberization is promoted by a relatively alkaline reaction in the sap, since the oxidation of fatty substances proceeds more rapidly on the alkaline side of pH 6.5.

Now the observations set out in the present and previous papers (8, 9) show that, given adequate suberization, wounded surfaces of the Gros Michel banana are not penetrated by the hyphae of *F. cubense*. Suberin deposition, however, is directly conditioned by a number of external factors of which oxygen supply and water relations are of first importance. It has been shown by controlled inoculation experiments, that where these conditions are adequately fulfilled infection does not take place, while ecological studies indicate that where the incidence of disease in the field is greatest, soils are poorly aerated or suffer from unfavourable water relations (7).

Once the stele of the sucker has been invaded by the hyphae of *F. cubense* the further spread of the parasite through the sucker appears to be inevitable. Structural modifications are also present in internal parenchymatous tissue, but nevertheless, the fungus slowly spreads through the vessels and the protective mechanisms which check the invasion in superficial tissues appear to be inefficacious once deeper penetration has taken place. This may be attributed to several factors, but on none have we any definite information.

VIII. DISCUSSION.

It is now well known that, as a result of genetic constitution, while some varieties of bananas are definitely immune to Panama disease, others such as the Gros Michel are susceptible. The problem before the pathologist was to determine whether this susceptibility was constant for all external conditions, or whether it might be possible to delay or prevent infection by modifying the adverse soil conditions under which the Gros Michel banana is frequently grown.

From the detailed observations accumulated during these studies the case for conditional infection may now be briefly discussed. The following facts have been ascertained :

1. Suckers grown in well aerated, moist soils, heavily inoculated with *F. cubense*, do not become diseased by way of the basal cut end or through superficial wounds, including old leaf-bases.

2. Such possible infection courts are only invaded by hyphae to a slight extent, deeper penetration being prevented by the development of suberized cambiform barriers. It has been amply demonstrated that the latter cannot be penetrated by the hyphae of *F. cubense*. These protective barriers formed in relation to wounding or to the diffusion of fungal secretions are the result of vital activities on the part of living parenchymatous tissues. The important external factors controlling such activities, i. e., cell-division and cork-deposition, include moisture and oxygen supply. Other factors may also be involved. Thus any external factors which militate against the rapid deposition of suberin and the growth and division of tissues will tend to promote infection. Water-logging or inadequate aeration at the time of wounding might be cited as examples of external conditions inimical to the formation of the characteristic defensive mechanisms.

3. With regard to the operation of such adverse factors it may be stated that many of the soils on which the cultivation of the Gros Michel has been attempted, especially untilled virgin soils, suffer from inadequate aeration and from adverse water relations (7).

4. Under conditions favourable to wound healing, vessels which have been cut through or injured are penetrated by hyphae to a slight extent only. The presence of wound secretions, together with a collapse of vessels induced by the active expansion of adjacent parenchyma, effectively close such infection courts.

5. Sucker infection appears to be principally of lateral origin, taking place either by way of the root-bases or through the agency of boring organisms. Leaving the latter aside, root-base penetration may therefore be regarded as the critical stage in the development of a significant infection of the sucker. This leads to a consideration of root infection.

6. It has been shown experimentally that roots grown in heavily inoculated soils which are well aerated and kept uniformly moist do not become diseased after forty days. This is true of acid, neutral, and alkaline soils.

7. Variation in the supply of soil moisture, such as that caused by the drying out of an open soil, or by the introduction of a plasmolysing agent, leads to root disease.

8. Such root disease may be slight or extensive. Where the shock sustained has not been excessive, the healthy portions of the root develop protective suberized cambiform barriers by which deep fungal invasion is prevented.

9. Inadequate soil aeration or carbon dioxide vitiation also predispose to root disease, probably in relation to the absence of adequate suberization.

Ecologically, it has been found that this factor is important in heavy and compact untilled virgin-clay soils, and in soils subject to water-logging.

10. Penetration of the sucker through exposed root-bases is, under favourable soil conditions, prevented by the secretion of wound gum, by vessel collapse, and the occupation of vessel cavities by tyloses.

11. Significant infection of the sucker takes place when external conditions have been such as to make for a marked amount of root disease close to the root-base. Under certain conditions the infection may follow the track of boring organisms.

12. Once the hyphae of *F. cubense* have reached the sucker-stele their further spread through the sucker appears to be inevitable.

When these observations are taken collectively the case for conditional infection of the Gros Michel by *F. cubense* appears to be definitely established, since under favourable soil conditions root disease does not take place, and all of the several infection courts can be protected against deep fungal penetration. Thus one might postulate that the ideal banana soil, even though inoculated with *F. cubense*, would, under suitable climatic conditions, support the Gros Michel banana in a wilt-free state. While such soils do exist, the majority of those on which the cultivation of the Gros Michel has been attempted show some defect in fertility, water relations being frequently unfavourable. The practical aspect of the problem, however, is concerned with broad results rather than detailed experimental considerations. Thus while strong evidence for conditional infection has been adduced, it remains to be seen whether adverse field conditions can be modified by simple means to permit of the functioning of the several protective mechanisms.

IX. SUMMARY.

1. Under favourable soil conditions, infection of the banana sucker does not take place through the cut basal end or through superficial wounds.

2. Infection has invariably been found to be of lateral origin, associated with diseased root-bases or with the cavities produced by boring organisms.

3. Vascular penetration at the cut basal end is prevented by the secretion of wound gum and collapse of vessels.

4. Under favourable conditions penetration of suckers by way of root-bases is prevented by the secretion of wound gum, collapse of vessels, and occupation of vessel cavities by tyloses.

5. The initial stages in sucker infection are described.

6. Further observations are submitted on the subject of root infection. It is shown that this may be promoted experimentally by partial plasmolysis of roots in the soil.

7. The experimental results are summarized and their relation to field conditions discussed.

In conclusion, the writer wishes to express his thanks to the Principal and Members of the College Staff for the kind assistance invariably extended to him during the course of these investigations. In particular, he is indebted to Professor H. R. Briton-Jones for his valuable discussion of mycological aspects of the problem at all times, and to Professor F. Hardy for much useful information on soils.

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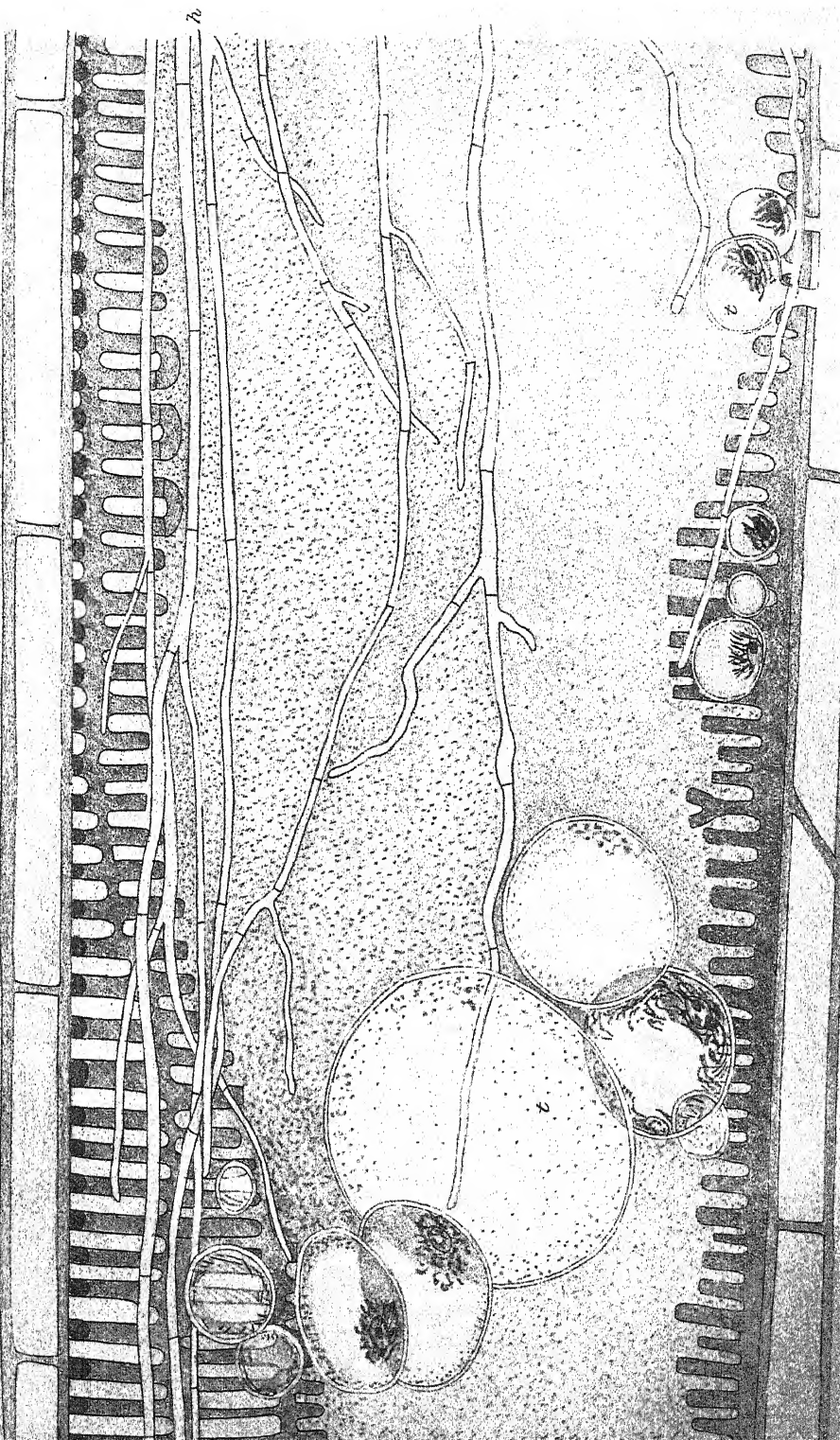
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DESCRIPTION OF PLATE XV.

Illustrating Dr. C. W. Wardlaw's paper on *The Biology of Banana Wilt*. Part III. An Examination of Sucker Infection through Root-bases.

PLATE XV.

Portion of a diseased vessel, showing the invading hyphae (*h.*) and the dead tyloses (*t.*). For description see text. × 350.



Studies in the Gramineae.¹

X. 1. Pennisetum, Setaria, and Cenchrus. 2. Alopecurus.
3. Lepturus.

BY

AGNES ARBER, M.A., D.Sc.

With eight Figures in the Text.

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¹ For references to previous papers in this series, which represents the work carried out with the aid of a grant from the Dixon Fund of the University of London, see 1-9 in the Literature cited, p. 419

1. *PENNISETUM*, *SETARIA*, AND *CENCHRUS*.

I. INTRODUCTION.

IN *Pennisetum*, *Setaria*, *Cenchrus*, and certain allied genera, we find bristles, or setae, associated with the spikelets; these bristles are either free, or united basally into a continuous involucre. They have commonly been described as sterile branches of the inflorescence, or sterile spikelets. With a view to obtaining, if possible, more critical evidence as to their nature, I have cut serial sections of the spikelets and associated structures in certain of these grasses. The bristles are apt to be highly sclerized and resistant to sectioning, so that I did not succeed in getting good preparations of all the species which I tried to examine. In the following pages those examples which gave the best results are described and illustrated.

I am indebted for material to the Director and to the Keeper of the Herbarium, the Royal Botanic Gardens, Kew; to the Director, s'Lands Plantentuin, Buitenzorg; and to the Director and to the Superintendent of the Cambridge Botanic Garden.

II. DESCRIPTION.

(i) *Pennisetum*.

Pennisetum, sp.

Fig. 1, A, p. 401, shows a partial inflorescence of a species of *Pennisetum* in which the spikelets occur singly amidst a cluster of bristles. Such a group, borne laterally on the main inflorescence axis, and including one or more spikelets with their associated bristles, may be conveniently called a *fascicle*. It will be noticed that one of the bristles is longer and broader than the rest; we will consider the meaning of this distinction when describing *P. macrourum*, Trin.

P. macrostachyum, Trin.

Fig. 1, B, illustrates the anatomical structure of one of the bristles of *P. macrostachyum*, Trin. It consists almost entirely of thick-walled sclerized tissue, in which a single vascular strand is embedded. In this species the individual bristles may exceed 4 cm. in length.

P. macrourum, Trin.

In *P. macrourum*, three types of fascicle, containing respectively one, two, and three spikelets, can be recognized in my sections. The two simpler forms may be considered first, although they are probably derived by reduction from the three-spikelet form.

Fig. 1, C₁–C₃, represents transverse sections from a series from below upwards through a young fascicle of the one-spikelet type. C₁ is near to

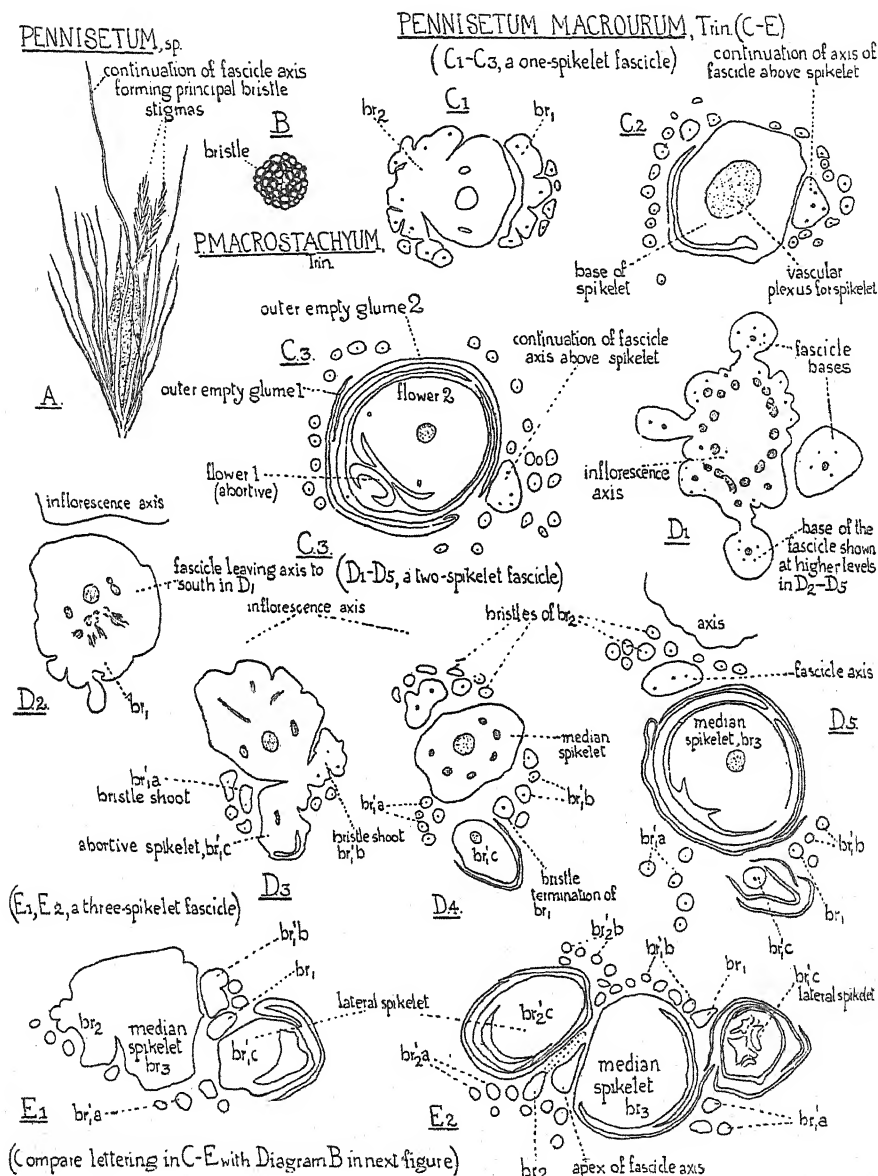


FIG. 1. *Pennisetum*. A, *P. sp.*, Buitenzorg Garden. Single fascicle; hairs borne by bristle omitted; spikelet dotted ($\times 3\frac{1}{2}$ circa). B, *P. macrostachyum*, Trin., Buitenzorg Garden. Transverse section of a bristle ($\times 193$). C-E, *P. macrourum*, Trin., Kew Gardens. Sections from transverse series through inflorescence. For explanation of the lettering see Fig. 2, diagram B, p. 405. C1-C3, a one-spikelet fascicle ($\times 47$). D1, axis with four fascicles detached, or in process of detachment ($\times 23$). D2-D4, sections at a higher level of the two-spikelet fascicle whose base is becoming detached to the south in D1 ($\times 47$); in D2, the abortive spikelet, *br'c*, and the median spikelet, *br's*, are shown. E1 and E2, two sections of a three-spikelet fascicle, E1 being below E2 ($\times 47$). In E2 the axis *br2* has curved so that its natural position is obscured. The dotted lines indicate its position at a slightly lower level.

the base, at a level at which one branch is partially, and one completely detached. These two branches are each in the act of branching again into a number of one-bundled bristles. This section shows that the bristles do not, as might be thought on superficial inspection, form a continuous wreath round the spikelet; they represent in fact, as Goebel has shown (12), the ultimate members of two branch systems, given off from the opposite faces of the fascicle axis. In Fig. C₂, a third branch—the spikelet—has been given off. Flanking the spikelet, a small object will be seen, larger than the bristles, but smaller than the adjacent spikelet base. This is the continuation of the main axis of the fascicle, which forms the single larger bristle, so often distinguishable in *Pennisetum* among the cluster of smaller bristles (Fig. 1, A).

In Fig. 1, C₂, the first outer glume is being detached from the spikelet base, while Fig. C₃ shows both the outer glumes, the lemma and the palea of the first flower, and the lemma of the second. The detailed structure of a flower from another spikelet is shown in Fig. 2, A₁ and A₂, p. 405. In this spikelet the first flower is undeveloped and is represented by its lemma alone. The second flower is, as usual, hermaphrodite. The lodicules are very small, and their aspect at this young stage suggests that they may be vestigial: most of the systematic accounts of the genus omit any mention of lodicules.

Fig. 1, D₁, shows the main axis of a young inflorescence, with one fascicle base just detached, and three others still connected with the axis, but about to become free. The fascicle to the south is of the two-spikelet type. Its history can be followed in D₂–D₅, which are drawn on a larger scale than D₁. The central spikelet and the continuation of the fascicle axis are seen in D₄ and D₅. In D₃ the base of the first bristle-shoot is coming into view, while in D₅ it is nearing detachment, and some of its bristles are already free. It differs from the corresponding shoot in C₁–C₃ in the fact that among the bristles an abortive spikelet (*br'*₁c in D₃–D₅) is given off laterally, while the shoot axis continues above it. The rachilla of the abortive spikelet bears three glumes, and continues above them as a slender organ, approximating in structure both to the one-bundled bristles and to the apex of the branch to which the spikelet is lateral.

The fascicle shown in Fig. 1, E₁ and E₂, included three spikelets—a median and two laterals. When the spikelets in a fascicle exceed one, we may regard the inflorescence branches of the second order as repeating the history of the whole of such a one-spikelet fascicle (inflorescence branch of the first order) as that shown in C. The lettering in Figs. E₁ and E₂ is intended to indicate this. For instance, the first branch, *br*₁ (inflorescence branch of the second order), bears two bristle-shoots (branches of the third order), *br'*₁a and *br'*₁b, while the abortive spikelet, *br'*₁c, forms the third branch of this order, and the axis *br*₁, which has borne these three succes-

side branches, continues above the spikelet. The same process takes place in the case of the second branch, br_2 , but here the order of events is a little

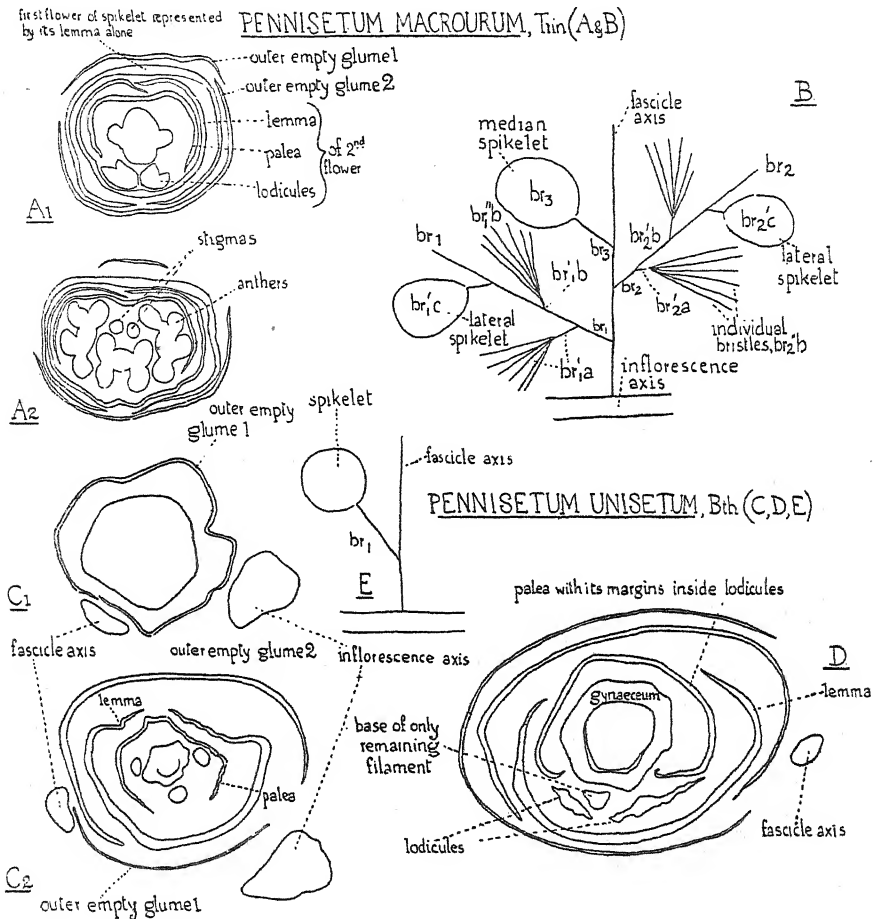


FIG. 2. *Pennisetum*. A and B, *P. macrourum*, Trin., Kew Gardens. A₁ and A₂, two sections, A₁, below, and A₂, above, through a young spikelet ($\times 47$). The first flower is represented by its lemma alone. B, diagram to explain the morphology of a three-spikelet fascicle such as that shown in section in Fig. 1, E₁ and E₂, p. 403. C-E, *P. unisetum*, Bth. C₁ and C₂, two sections, C₁ below, and C₂ above, from a transverse series through a fascicle from Tanganyika Territory, showing the inflorescence axis, and the bristle representing the fascicle axis, to which the spikelet is lateral ($\times 77$ circa). D, transverse section of spikelet from Nyasaland with an old flower, in which two stamens have disappeared, showing margins of palea inside lodicules ($\times 77$ circa). E, diagram of fascicle.

obscured by some displacement of the spikelet (br'_2c) in relation to the termination of the axis to which it is lateral (br_2). The median spikelet is the third inflorescence branch of the second order (br_3), while the inflorescence branch of the first order (the termination of the axis of the whole fascicle) continues above it. I have added a purely diagrammatic

figure (Fig. 2, B, p. 405) to explain my idea of the relations of the branch systems in a three-spikelet fascicle, such as that shown in Fig. 1, E_1 and E_2 .

In the series shown in Fig. 1, C_1 – C_3 , which has one spikelet, it is impossible to say with any certainty whether any one of the bristles of either bristle-shoot is terminal to the branch system to which it belongs. It is only when a spikelet is developed as the uppermost branch that the apex of the axis becomes distinguishable. Moreover I have found it impossible, from a study of serial sections, to determine whether the individual bristles in any bristle-shoot are all of the same order. The main axis of each bristle-shoot is highly abbreviated, and it breaks up into component bristles in a way that defies reduction to a definite scheme of branching.

• *P. ciliare*, Link (*P. cenchroides*, Rich.).

I have cut serial sections of herbarium material of fascicles of *P. ciliare* from Angola and from Uganda. Their hardness makes it difficult to get good preparations, but the general structure of a two-spikelet fascicle is shown in Fig. 5, C_1 and C_2 , p. 411. This species is remarkable for the fact that the bristles are united for a short distance at the base, thus foreshadowing the involucre of the related *Cenchrus* (p. 408).

P. unisetum, Bth., *P. nubicum*, (Hochst.) Chiov., and *P. petiolare*, (Hochst.) Chiov.

These species form a contrast to those just described, since the fascicle includes one bristle only, which is the termination of the fascicle axis (Fig. 2, C, p. 405). They agree also in the fact that the margins of the palea are curved inside the lodicules (Fig. 2, D). Trinius (16) mentions this peculiarity as characteristic of *Pennisetum*; I have found it also in *Setaria* (Fig. 3, B_3 , p. 407).

(ii) *Setaria glauca*, Beauv.

Fig. 3, A, p. 407, shows a spikelet of *Setaria glauca* with its associated bristles, belonging to two branch systems. These fascicles, as in *Pennisetum*, are borne directly upon the inflorescence axis. They usually contain one spikelet. The nature of the one-spikelet fascicle will be understood from the series of sketches B_1 – B_6 . Fig. 3, B_1 , shows the axis of an inflorescence and the bases of four fascicles, the highest (*fascicle* 4) not yet having become free. Each fascicle base contains three main bundles. In B_4 , *fascicle* 3 is seen at a higher level. It has divided into three branches—the median one is the stalk of the spikelet, while the axes to right and left, br_1 and br_2 , bear bristle-shoots; that to the left is cut obliquely, and demonstrates the alternate arrangement of the bristles. B_5 , which is cut at a higher level, shows the median spikelet. There are two outer empty

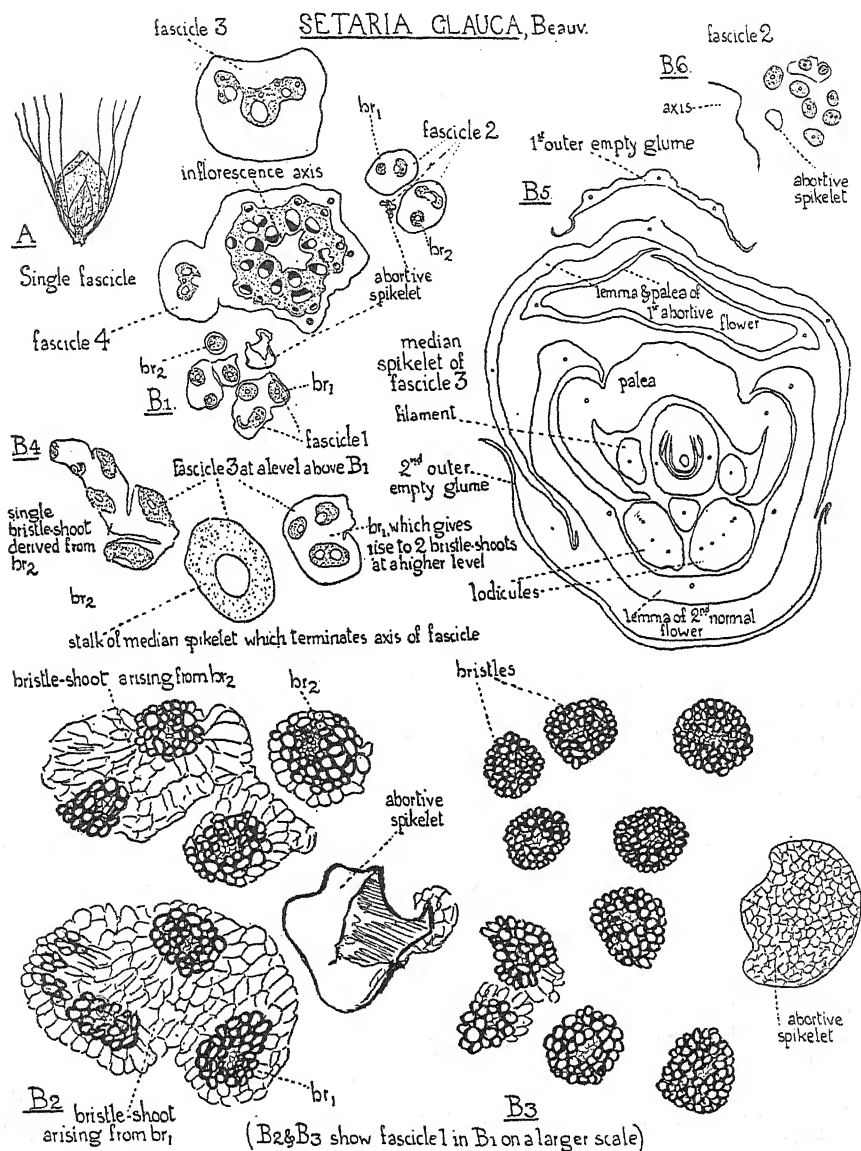


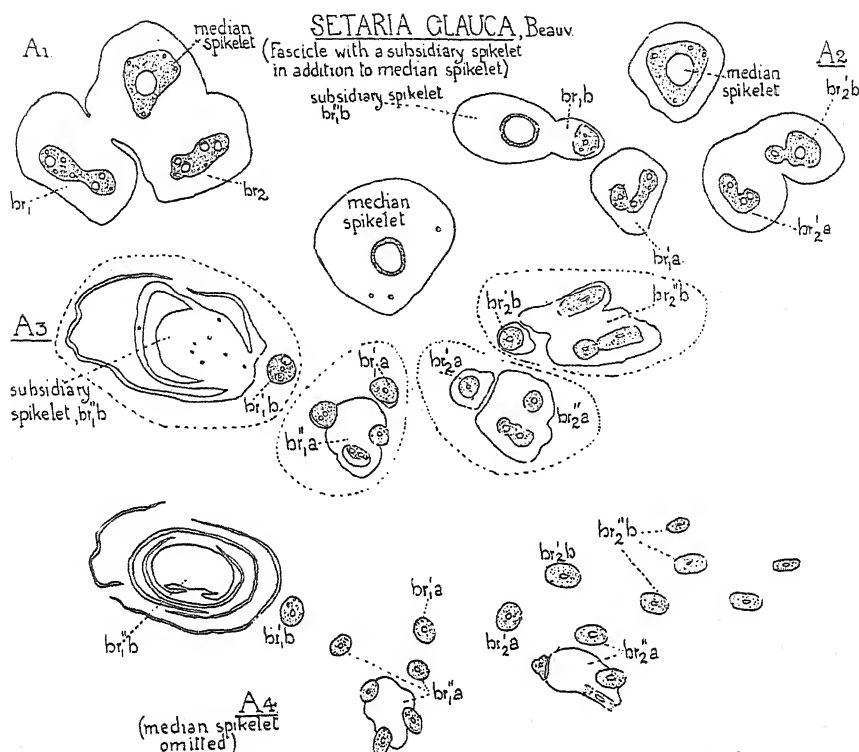
FIG. 3. *Setaria glauca*, Beauv., Cambridge Botanic Garden. A, fascicle consisting of a one-flowered spikelet with accompanying bristles (\times rather less than three). B₁–B₆, sections from a transverse series cut near the base of an inflorescence. B₁, axis with the bases of fascicles 1–4; 4 is not yet detached ($\times 47$). B₂ and B₃, fascicle 1 at level of B₁ and at a higher level ($\times 193$ circa); it is not possible to be certain that all the bristles drawn belong to this fascicle; the orientation is not exactly the same as in B₁. B₄ and B₅, sections through fascicle 3 at levels above B₁ ($\times 47$). In B₅ only the median spikelet is shown; the bundles are omitted from the second outer glume, which is poorly preserved. B₆, fascicle 2 at a higher level ($\times 47$).

glumes, followed by the flowering glume (lemma) and palea of the first flower, which is itself abortive, and is represented only by a minute rudiment above the palea. The second flower is hermaphrodite. In one small point the ground-plan is unlike the usual grass type, but agrees with *Pennisetum unisetum* (Fig. 2, D, p. 405); the margins of the palea turn inside the lodicules instead of enclosing them. The whole shoot apex of the spikelet is used up in forming the two flowers with their lemmas and paleas; there is thus no rachilla left between them. *Fascicles* 1 and 2 differ from the fascicle just described in that the spikelets which they include are abortive instead of normal. In Fig. 3, B₂, *fascicle* 1 in Fig. 3, B₁ is drawn on a larger scale to show the abortive spikelet and the two branches *br*₁ and *br*₂, each giving off a bristle-shoot. The same group is drawn in B₃ at a higher level at which all the bristles have become free. B₆, again, shows *fascicle* 2 at a higher level than in B₁, with the setae of the two groups almost all detached.

Though it is usual for *Setaria glauca* to show one spikelet only in the fascicle, occasionally a second may be found. I have cut serial sections through three fascicles in which there was a subsidiary spikelet in addition to the main spikelet. One of these is illustrated in Fig. 4, A₁–A₄; the two other series, though less perfect, gave confirmatory evidence. The system of branching is not easy to follow: my interpretation of it may be understood from the diagram B, Fig. 4, p. 409. The median spikelet terminates the axis. Below this, two branches, *br*₁ and *br*₂, are given off. One branch, *br*₂, produces two laterals, *br'*₂*a* and *br'*₂*b*, but does not itself continue above their point of origin. Two bristle-shoots, *br''*₂*a* and *br''*₂*b*, are formed as lateral branches of *br'*₂*a* and *br'*₂*b*. The other main branch, *br*₁, also gives two branches of the second order, *br'*₁*a* and *br'*₁*b*, and disappears itself in doing so. One of these branches, *br'*₁*a*, bears a bristle-shoot, *br''*₁*a* laterally; the other, *br'*₁*b*, bears a spikelet, *br''*₁*b* laterally. If my interpretation is correct, the four groups of members enclosed within dotted boundaries in Fig. 4, A₃, may be regarded as equivalent. We will consider the meaning of this in the Discussion (p. 412).

(iii) *Cenchrus*.

The inflorescence axis of *Cenchrus* resembles that of *Pennisetum* and of *Setaria* in bearing a series of fascicles, but in these fascicles the spikelets, instead of being associated with a group of independent bristles, are enclosed in an involucre consisting of bristles in a state of more or less complete union. As has been already mentioned, *Pennisetum ciliare*, Link (*P. cenchroides*, Rich.) is intermediate in involucre construction between *Pennisetum* and *Cenchrus* (Fig. 5, C₁ and C₂, p. 411).



(B, diagram to show scheme of branching for the fascicle seen in section in A₁-A₄)

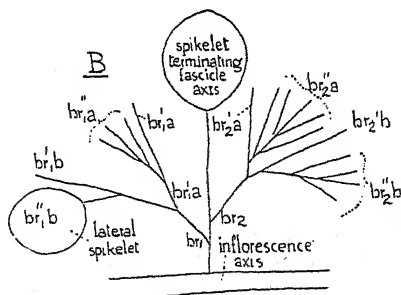


FIG. 4. *Setaria glauca*, Beauv. A₁-A₄, series of transverse sections ($\times 47$) through a fascicle with a subsidiary lateral spikelet in addition to the central spikelet. B, diagrammatic scheme of the mode of branching of the fascicle illustrated in A₁-A₄. For further description see text, p. 408.

Cenchrus inflexus, R.Br.

Fig. 5, A, p. 411, shows the abaxial face of one of the fascicles of *Cenchrus inflexus*, with its spiny involucre. A series of sections which I cut through, one such fascicle showed a structure closely resembling that of *C. echinatus*, described below.

C. echinatus, L.

Serial sections through the fascicle of *C. echinatus* reveal either one, two, or three spikelets. Fig. 5, B₁–B₄, illustrates a one-spikelet fascicle. In B₁ the base of the fascicle is shown, with the axis from which it arose. It will be noticed that there is a central vascular core destined for the spikelet, while the surrounding strands are differentiated into a series which are passing out to the right, and a second series to the left, which are still vertical. In front there is no sharp division between these series, though at the back there is a gap between them. In Fig. B₂ the involucre has separated from the spikelet, and the difference between the development of the bristles on the two sides, foreshadowed by the anatomy of Fig. B₁, confirms Goebel's interpretation of the involucre as consisting of two lateral branch systems (12). So far as can be judged, the spikelet is terminal to the fascicle axis, as in *Setaria*. The spikelet, which is enclosed by only one outer empty glume, includes two flowers; the lower is male with a rudimentary gynaeceum, which does not reach to the level of Fig. B₃. There is no rachilla between the flowers, and the flowers contain no lodicules. In Fig. B₄ the stigmas and anthers of the upper flower are cut at a higher level, and the involucre is seen in its more divided upper region.

Fig. 6, A₁–A₃, p. 412, illustrates a two-spikelet fascicle, marked *fascicle 1*, which in B₁ is just detached from the inflorescence axis. It has two main bundles. Its actual attachment to the axis is not visible in my sections, but in *fascicle 2* (another two-spikelet fascicle), which is just leaving the axis in Fig. A₁, it can be seen that a pair of main bundles enter at the base. Fig. A₂ is higher, and shows that the two main bundles of *fascicle 1* were each destined for a spikelet. It will be seen that the involucre at this level does not merely enclose the spikelets, but extends between them. This suggests that the involucre here may have a complex character similar to that of the two-spikelet fascicles of *Pennisetum macrourum* and of *Setaria glauca* previously described.

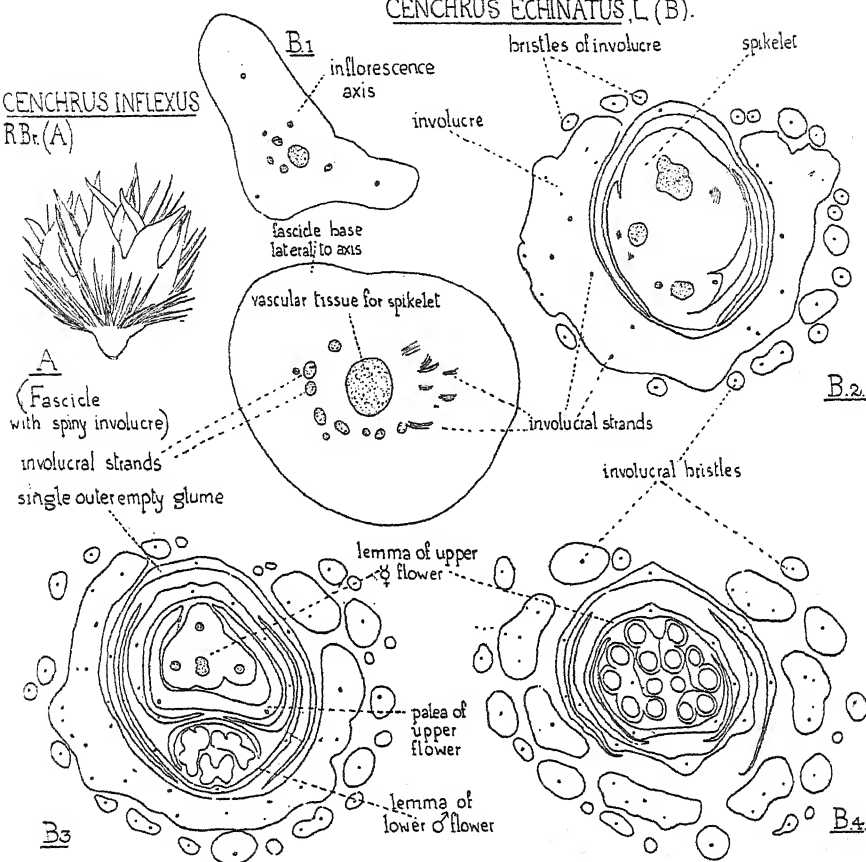
In Fig. 6, B₁–B₃, p. 412, which represents a three-spikelet fascicle, two bundles enter the base of the fascicle (B₁) but one immediately branches, so that there is a triad, each member of which supplies a spikelet (Figs. B₂ and B₃).

C. myosuroides, H. B. et K.

I have cut sections of two dried fascicles of this species from Florida. They were each one-flowered and closely recalled a one-flowered fascicle of *C. echinatus*. The construction was: two outer empty glumes; a flowering glume representing the lower flower; hermaphrodite upper flower with no lodicules. In the base of the fascicles, the bundles for one half of the involucre pass out distinctly earlier than those for the other half, and the

CENCHRUS ECHINATUS, L. (B).

CENCHRUS INFLEXUS

$$RB_r(A)$$


PENNISETUM CILIARE (L) Link (P. CENCHROIDES, Rich.) (C)

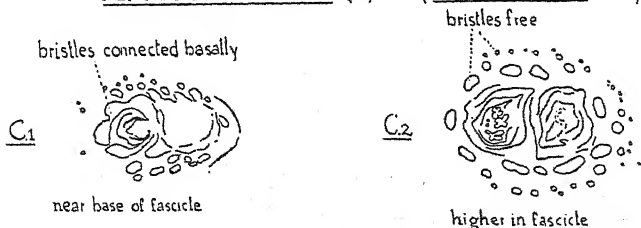


FIG. 5. *Cenchrus*. A, *C. inflexus*, R. Br., Buitenzorg Garden, April 8, 1929. Single fascicle viewed from the abaxial side to show the involucre ($\times 3\frac{1}{2}$ circa). B, *C. echinatus*, L., Cambridge Botanic Garden. B₁-B₄, transverse sections from series upwards from below through a young one-spikelet fascicle ($\times 47$). B₁, base showing vascular strands for involucre and spikelet. B₂-B₄, spikelet and involucre; B₃ shows the two flowers, of which the lower is male. Lodicules and first outer empty glume absent. B₄, involucre largely subdivided into bristles. C₁ and C₂, *Pennisetum ciliare*, Link (*P. cenchrroides*, Rich.) from Angola. Two transverse sections, C₁, below, and C₂, above, through a fascicle to show the bristles partially fused basally in C₁, and free in C₂ ($\times 14$).

evidence that the involucre consists of two opposite branch systems is even clearer here than in *C. echinatus*.

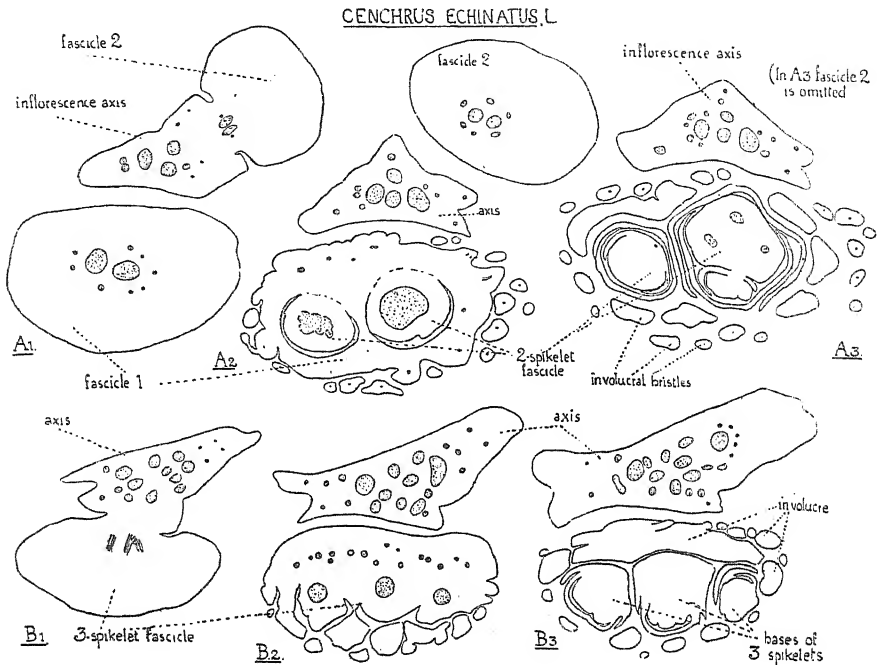


FIG. 6. A and B, *Cenchrus echinatus*, L., two sections from a series from below upwards through a young axis and fascicles. A₁–A₃, a two-spikelet fascicle (*fascicle 1*) $\times 36$ circa. In A₁, *fascicle 1* is detached from the axis; another (*fascicle 2*), which is cut at the level of detachment, has two bundles at the base. B₁–B₃, a three-spikelet fascicle; B₁, base; B₃, higher level, passing through three spikelets.

III. DISCUSSION.

Hofmeister (13) regarded the bristles in *Setaria* and *Pennisetum* as the stalks of abortive spikelets, and Goebel (12) appears to associate himself with this theory, though I am not sure that I exactly understand his opinion. Schumann (14) calls the bristles 'spikelet stalks or infertile axes of the last order', while other authors have used vaguer expressions, such as 'sterile inflorescence branches'. None of these views seem to me to conform precisely to the facts. My interpretation of the nature of the branching in the fascicle of *Pennisetum macrourum* and of *Setaria glauca* will be understood from Fig. 2, B, p. 405, and Fig. 4, B, p. 409. But I want to make it clear that this interpretation is of a tentative character, and relates only to the species in question. A full comparative investigation of a large number of species by means of serial sections is much needed. Until this has been undertaken, no generalized scheme for the fascicle of *Pennisetum* and *Setaria* can be justified. My present study of these two genera has led

me to the provisional view that *each ultimate bristle-shoot*—not each individual bristle—is *equivalent to a spikelet*. Hofmeister (13) speaks of the occurrence of bristles bearing glumes, but he does not describe their exact relation to the rest of the fascicle. I should expect that they would be found to be examples such as that shown for *Pennisetum macrourum*, in Fig. 1, D₅, p. 403, in which an abortive spikelet is given off as the third branch of the first lateral branch of the fascicle axis. If this be so, these reduced spikelets do not prove the equivalence of an individual bristle and a spikelet.

I think that the relations of the parts of the branch system are best understood from such a fascicle as that of *Setaria glauca*, shown in Fig. 4, A₁–A₄, p. 409. Here the two lateral branches, arising below the terminal spikelet, are seen to give rise to four branch systems, each of which is encircled by a dotted line in Fig. 4, A₃. In each case there is a terminal bristle axis (br'_1a ; br'_1b ; br'_2a ; br'_2b). A spikelet (br''_1b) is lateral to br'_1b , while three bristle-shoots (br''_1a ; br''_2a ; br''_2b) are lateral to the three other bristle-axes (br'_1a ; br'_2a ; br'_2b). It follows that the spikelet br''_1b , and the bristle-shoots br''_1a , br''_2a , br''_2b , are morphologically equivalent. As the spikelet axis does not branch again, whereas the bristle-shoot becomes subdivided into numerous setae, I do not think we can equate the *individual* bristles with any part of the fertile shoot; we cannot go beyond the equivalence of the lateral bristle-shoot, regarded as a whole, and the spikelet.

The individual bristle of *Pennisetum*, *Setaria*, and *Cenchrus* is simple in construction and frequently one-bundled (Fig. 1, B, p. 403, Fig. 3, B₃, p. 407). It might, as far as structure is concerned, be equally well interpreted as of leaf or stem nature. It forms one of the many illustrations that might be cited to demonstrate the artificiality of the morphological distinction between stem and leaf.¹ We can find terms of comparison for the individual bristle in both these categories among the grasses. For instance, the bristle-like glumes of *Hordeum*, which are leaf members, may be cylindrical and one-bundled (e.g. *H. pratense*, Huds., 7, Fig. 2, B₆, p. 510), while the same structure recurs in the rachilla (axis) of the sterile spikelet in *H. distichum*, L. var. *nigrum* (7, Fig. 3, B₃, p. 515). But it is probably best for descriptive purposes to treat the bristles of *Setaria*, *Pennisetum*, and *Cenchrus* as stem structures, since certain bristles in *Setaria* (e.g. br'_1a ; br'_1b ; br'_2a ; br'_2b , in Fig. 4, A₄, p. 409) play the part of axes in relation to lateral members; and also because the axis of an abortive spikelet may be bristle-like (e.g. *Pennisetum macrourum*, br'_1c , Fig. 1, D₅, p. 403). In *Pennisetum*, moreover, a single bristle larger than the rest is often distinguishable by the naked eye. This bristle, which is seen in Fig. 1, A, p. 403, is the continuation of the fascicle

¹ See (10) for a further consideration of this point.

axis to which the spikelet is lateral.¹ In such species as *P. unisetum*, Bth., it is the only bristle formed (*fascicle axis*, Fig. 2, C-E, p. 405). In his account of the fascicle of *Pennisetum*, Goebel (12) seems to have overlooked the fact that the axis continues in this way beyond the spikelets. Unlike that of *Pennisetum*, the median spikelet of *Setaria glauca* terminates the fascicle axis, although when subsidiary spikelets occur the axis to which they are lateral continues beyond them. My sections fail to confirm Goebel's diagram (12, Pl. I, Fig. 18), showing what he describes as a 'wicklige Verzweigungssysteme' in the fascicle of *Setaria*. On the contrary, what appears to be alternate and non-cymose branching of a bristle-shoot may be recognized in Fig. 3, B₄, p. 407. But this point requires further investigation.

My observations confirm Goebel's view that the involucre of *Cenchrus* can be analysed into two lateral branch systems, equivalent to those which form the independent branches of *Pennisetum* and *Setaria* (Fig. 3, A, p. 407). This point is specially clear in *Cenchrus myosuroides*, H. B. et K. The absence of lodicules in *C. echinatus* and *C. myosuroides*, and the absence of one outer glume in *C. echinatus*, may be correlated with the pressure exercised by the conerescent involucre.²

2. *ALOPECURUS*.

The apparent spike of *Alopecurus pratensis*, L., is well known to be a complex though condensed inflorescence. Goebel (12) has interpreted it, from a study of young stages, as essentially dorsiventral. The inflorescence axis bears a crowded series of spikelet-fascicles. I followed serial sections of a 'spike' through the origin of seven fascicles. Among these fascicles I noticed the occasional occurrence of structures which seemed to be extremely reduced rudimentary leaves borne upon the inflorescence axis (Fig. 7, A₁, p. 415). As a rule each fascicle was composed of three branchlets. The fascicle was either detached as a single organ, which did not divide until after it had become free, or one branchlet became separated while the others were still united with the axis. Fig. 7, A₁, p. 415, shows a fascicle, consisting of three adjacent branches, *x*, *y*, and *z*, arising at approximately the same level from the main axis of the 'spike' and fused at their bases. The branch *x*, which is shown detached in A₂, has two main bundles and two minor ones. It forks into two spikelet axes, *x'* and *x''*. Fig. A₃ shows *x'* after it has separated from *x''*; its minor bundle has vanished by the dying out of its phloem and the fusion of its xylem with that of the main bundle, so that the spikelet axis has now one collateral

¹ It is therefore not quite correct in a morphological sense to describe this bristle, as has been done in systematic work, as 'the branchlet at the base of which the spikelet . . . is borne' (11).

² For Summary, see p. 418, § 1.

bundle only. Its twin axis, x'' (not shown in the diagrams), retains its two bundles rather longer than x' , but they, also, become reduced to one below the base of the spikelet; in this case both the xylem and phloem of the

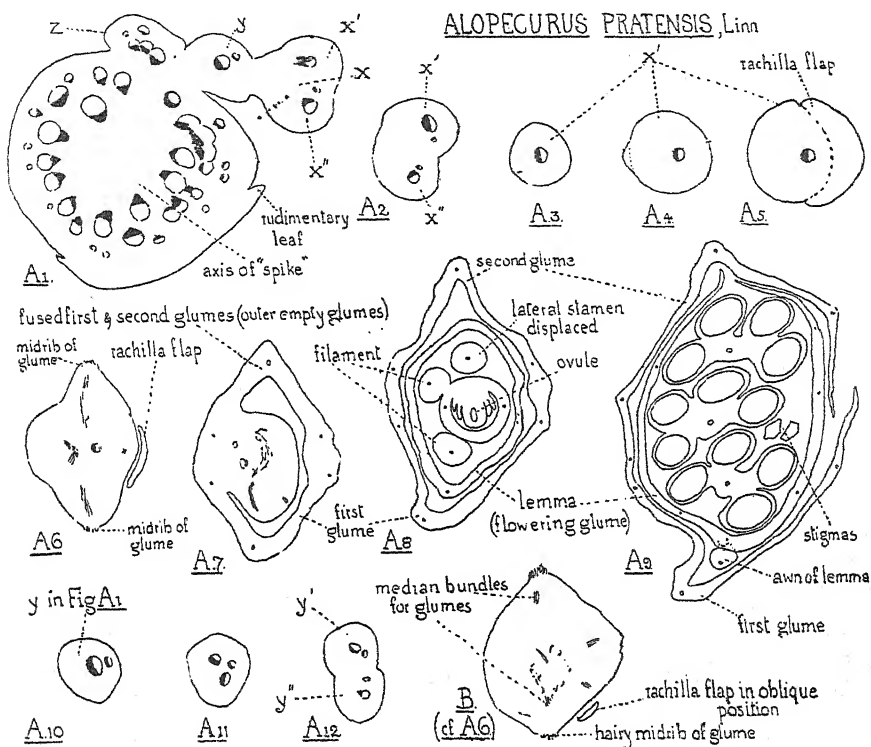


FIG. 7. *Alopecurus pratensis*, L. A₁–A₁₂, sections from a transverse series from below upward through a segment of a 'spike', May 6 ($\times 47$). A₁, axis of 'spike' showing base of a fascicle of three branches, x , y , z . Fig. A₂, branch x just about to divide into x' and x'' . Figs. A₃–A₅, the further history of x' ; A₈ passes through the ovule and A₉ through the anthers. Figs. A₁₀–A₁₂, the further history of branch y , which is shown attached in Fig. A₁. Fig. B, transverse section of the base of another spikelet ($\times 47$) to show a case in which the rachilla flap is placed obliquely.

smaller bundle become incorporated with those of the main bundle. The branch y in Fig. A₁ is seen in A₁₀ above its detachment. Its two bundles subsequently become four (A₁₂), and it divides into a pair of two-bundled branches, y' and y'' . When y' is followed up, it is seen that the xylems of the two bundles fuse and the phloem of the smaller bundle dies out, leaving one collateral strand. In y'' , again, we get a reduction to one bundle by fusion of the two. The third branch, z , in Fig. A₁, gives rise to seven axes, which probably all terminate in spikelets, though I have only been able to follow four of them as far as the spikelet itself. It thus appears that the three branches, x , y , z , give rise to eleven one-bundled axes, each terminating in a single-flowered spikelet.

The history of the spikelet x' can be followed in A_3 to A_9 . In A_4 the prominence seen to the left is the first indication of the oblique 'articulation' below the spikelet. As we pass successive sections in review, the boundary between the prominence and the rest of the tissue moves gradually to the right, and in A_5 the excrescence has engulfed the bundle—in other words, the bundle has entered the segment of the axis above the articulation. Finally, in A_6 , the margin of the tissue below the joint stands up to the right of the section as a free tongue—the rachilla-flap; this structure has been discussed in an earlier paper (3). In x' , x'' , y' , and y'' , and in six other spikelets which I examined, the rachilla-flap was found to occur at the side of the axis remote from the xylem face of the collateral bundle; in seven cases it was directly opposite the xylem, and in the remaining three it was obliquely placed. Above the articulation we come to the first two glumes (outer empty glumes). Their midribs usually arise in symmetrical fashion from either side of the single collateral bundle of the spikelet axis, and hence the plane of their midribs is parallel to the face of the rachilla-flap (Fig. 7, A_6). But this relation is not invariable, and the midrib plane may be obliquely placed with regard to the rachilla-flap (Fig. B). It is curious that articulation of the reproductive axes, though it is an anomalous feature common to many grasses, seems to have eluded morphological explanation.

The two first glumes of *Alopecurus pratensis* are fused at the base into a closed structure, and the lemma (flowering glume) has its margins united in the basal region (Fig. 7, A_8). The glumes thus form a closed case, which is narrower from side to side than it is in the antero-posterior plane. Hence the flower develops under some lateral pressure, and with this compression we may perhaps associate several of the peculiarities of the spikelet—absence of any rudiment of the rachilla above the glumes (15); absence of palea; absence of lodicules; and displacement of one of the lateral stamens into the posterior position (Fig. 7, A_8 and A_9). This displacement gives the flower a curiously asymmetrical appearance when seen in section. In Fig. A_7 the lateral attachment to the glumes of the mass which will differentiate into lemma, stamens, and pistil, is normal, and not, as might be supposed, an effect of obliquity in the section. The stamen which retains its lateral position is attached at one side of its base to the gynaeceum and at the other to the lemma, while at the extreme base this fusion involves the outer glume also.¹

3. LEPTURUS.

The genus *Lepturus*, R. Br. (*Rottboellia*, Host), which is assigned to the Hordeae, is remarkable for the deep excavations in the inflorescence axis in which the spikelets are sunk. Goebel (12) has recorded some

¹ For Summary, see p. 419, § 2.

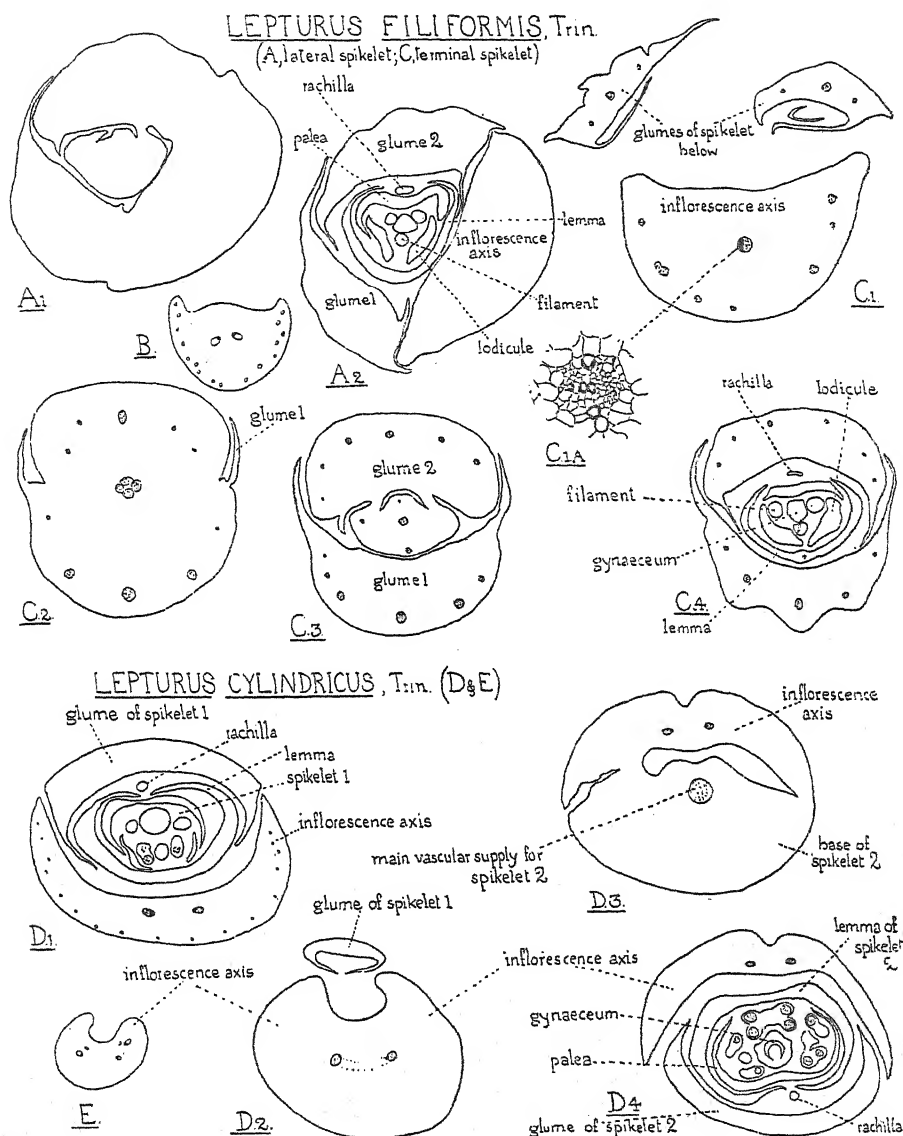


FIG. 8. *Lepturus*. A-C, *L. filiformis*, Trin. A₁ and A₂, transverse sections, A₁, lower, A₂, higher, from a series through a lateral spikelet ($\times 47$). B, transverse hand section through another inflorescence axis below a lateral spikelet ($\times 23$). C₁-C₄, transverse sections ($\times 47$) from a series from below upwards through a spikelet whose structure shows it to be terminal. C₁ (drawn from more than one section), axis below spikelet; C₁A, the bundle in C₁ ($\times 193$). C₂, spikelet-base. C₃, flower. D and E, *L. cylindricus*, Trin. D₁-D₄, transverse sections from a series from below upwards through a segment of an inflorescence axis bearing two spikelets ($\times 47$). Except the two principal strands, the bundles in the inflorescence axis are too embryonic to be shown exactly, but the general scheme is as indicated in D₁. E, transverse section of an inflorescence axis, with unignified bundles omitted ($\times 23$).

observations on the genus, but apparently without using sections. I have cut serial sections of the 'spikes' of two species, *L. filiformis*, Trin., and *L. cylindricus*, Trin. Fig. 8, A₁, p. 417, passes through the base of a lateral spikelet, while in A₂ the spikelet itself is reached. The outer glumes occupy an unusual position, appressed to the axis. There is a single flower, and the rachilla continues for a short distance above its base. Fig. 8, C₁–C₄, gives the history of a terminal spikelet. Below it the inflorescence axis has a single collateral main bundle placed sideways (Fig. 8, C₁A); I have seen a corresponding single bundle in the axis below another terminal spikelet. The outer empty glumes face one another, since the inflorescence axis does not continue above the base of the last spikelet, and hence there is no obstacle to force the glumes to one side, as in the lateral spikelet (A₂). Figs. 8, D₁–D₄, are from a series passing upwards through two successive spikelets of *L. cylindricus*, Trin. Fig. 8, D₁, shows that this species has a single outer glume instead of the usual pair. The inflorescence axis is flattened and leaf-like, with two main bundles and a number of minor ones. A little higher a bridge connects the two main bundles (D₂), and, from this bridge, bundles for the main vascular supply of the spikelet are given off, while the two main strands continue into the next segment (D₃). The next spikelet is shown in D₄.

The chief morphological interest of the genus lies in the parallel it affords to *Hordeum* in the relation of glumes and axis. The symmetrical position of the glumes in the terminal spikelet of *L. filiformis* (Fig. 8, C₄), proves that their lateral position in the non-terminal spikelets (Fig. 8, A₂) is imposed upon them by the flattened inflorescence axis to which they are appressed. The relation of glumes to axis recalls that in the main spikelet of *Hordeum distichum*, L. var. *nigrum*, figured in 7, Fig. 3, A₆, p. 515.

Another point to which attention may be called, is that the inflorescence axis of *Lepturus* shows how closely a purely axial member may mimic a foliar member.¹ Fig. 8, B, has very much the look of a petiole section, while the inflorescence axis in D₁ or D₄ might be taken for a section of a binerved palea or prophyll, but for the fact that the xylem of the two main bundles is lateral instead of ventral.

4. SUMMARY.

The three sections into which this paper is divided may be summarized as follows:

I. After description of the fascicles which form the units of the inflorescence in *Pennisetum* (pp. 403–6 and Figs. 1, 2, and 5, C), *Setaria* (pp. 406–8 and Figs. 3 and 4), and *Cenchrus* (pp. 408–12 and Figs. 5 and 6),

¹ For a consideration of the meaning of such cases, see (10).

the morphological interpretation of these structures is considered. A suggested explanation is indicated diagrammatically in Fig. 2, B, p. 405, for *Pennisetum macrourum*, Trin.; Fig. 2, E, p. 405, for *Pennisetum uni-setum*, Bth.; and Fig. 4, B, p. 409, for *Setaria glauca*, Beauv. It is concluded that *the ultimate bristle-shoots are equivalent to spikelets*; for instance, the subsidiary spikelet br''_1b , seen in Fig. 4, A₄ and B, p. 409, is equivalent to the bristle-shoots br''_1a , br''_2a , br''_2b , seen in the same figures.

2. The structure of the fascicles of spikelets which make up the 'spike' of *Alopecurus protensis*, L., is described (pp. 414-16 and Fig. 7). It is shown that the glumes, lemma, androecium, and gynaeceum are all supplied from a single collateral bundle in the stalk of the spikelet. The absence of the rachilla apex, palea, and lodicules, and the asymmetry of the flower, are ascribed to the fusion of the outer empty glumes, and the tunication of the lemma at the base, which cramp the flower in its development.

3. The structure of the spikelets of *Lepturus*, and their relation to the inflorescence axis, are described and figured (pp. 416-18 and Fig. 8). In their anomalous lateral position, the outer empty glumes of *L. filiformis*, Trin., may be compared with those of *Hordeum*; they owe this position to their close association with the dorsiventral main axis of the inflorescence, which impedes their development on one side.

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Observations on the Action of Bromine on Plant Tissues.

BY

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IN the continuance of researches on the chemical nature of the cell membrane in plant tissues (1), when a reaction for the detection of protein in the cell-wall was employed which involved the use of chlorine gas and subsequent treatment of the material in the form of sections with sodium hydrogen phosphate and potassium iodide solutions, certain definite colour effects were produced. It was found that a pink coloration developed in lignified tissues during this reaction (2), and while this effect was under investigation, it was decided to find out what result the substitution of bromine for chlorine would have upon such material, as an analogous compound should be formed, and possibly the method might be simplified further. To this end sections of the plant material were exposed to the action of bromine vapour, while for purposes of comparison, others of the same kind were exposed to the action of chlorine gas in the apparatus designed by the author, and described in a previous paper (1), when applying the chloramine reaction for protein to sections of plant material.

The use of chlorine and bromine in precisely this manner, as far as the author is aware, is unrecorded elsewhere.

When using bromine, the sections were placed in small bags made of 'superfine Tarantulle', which had been previously exposed to the action of bromine vapour for a considerable period, and then rinsed thoroughly in water.

The bags were suspended in a desiccator containing liquid bromine in the lower part, instead of the usual dehydrating agent, for periods varying from eighteen to twenty-four hours, according to the penetrability of the material to the vapour. The process took much longer than when chlorine gas was used, since in the latter case a stream of the gas was passed through the sections (1), while in the former case the bromine vapour slowly disseminated through them, thus considerably increasing the time factor,

although the lid was not placed upon the desiccator until it was filled completely with bromine vapour.

Plant material which gave a strong pink coloration after treatment with chlorine was used, viz. stems of *Cycas revoluta*, *Larix europaea*, *Taxus baccata*, *Pseudotsuga taxifolia*, and *Bambusa* sp.

After bromination the bags containing the sections were washed in water—a duplicate set being prepared—one set being washed in cold water alone, and the other subsequently washed with hot water. The latter operation was performed so as to destroy any trace of oxonium salt which causes the sections to become a dark colour.

In both cases the xylem appeared dull orange in colour, the depth of colour being considerably reduced, and the sections made more transparent after treatment with hot water. A large number of sections were examined, and repeated experiments on transverse sections of the stems of these plants performed, with the following results on the various tissue systems:—

(a) *Sclerenchyma*.

Taxus baccata was the only specimen examined providing marked sclerenchymatous tissue giving the pink coloration. *C. revoluta* gave a dark brownish-red colour when bromine was used, which was a clear red when the sections were treated with hot water before submitting them to the action of sodium hydrogen phosphate and potassium iodide. *T. baccata* gave an orange colour after cold and after hot water treatment, while *Bambusa* sp. gave a dark red coloration, appearing orange-red after the hot water treatment.

(b) *Xylem*.

In all cases a decided pink colour was produced after treatment with chlorine, the autumn xylem producing the deepest colour.

With bromine the colour varied from dark brownish-red (*Cycas*, *Larix*) to clear dark red (*Pseudotsuga*, *Taxus*, *Bambusa*) after cold water treatment. Washing with hot water did not alter the coloration, produced in *C. revoluta*, but in the other cases the brownish coloration did not appear.

(c) *Cortical tissues*.

There was no colour effect on the cell-walls of the cortical tissues when chlorine was used.

With bromine the effects were varied. There was no coloration in *C. revoluta*. *L. europaea* and *Bambusa* sp. both gave a bluish colour when treated with sodium hydrogen phosphate after washing in cold water, changing in the first case to dark blue, and in the second, to purple in the potassium iodide solution. In the former case hot water treatment effected no alteration in the coloration, while in the latter the cortex was dark

blue in the phosphate solution and became nearly black in the potassium iodide.

The results with *T. baccata* and *P. taxifolia* after hot and cold water treatment were alike, viz. an orange coloration in the cortical cell-walls.

Bromine evidently gives a compound analogous to that produced by chlorine when the material is treated subsequently with sodium hydrogen phosphate and potassium iodide, as in the chloramine reaction. That this is so points to the fact that the pink colour is associated with the presence of the halogen and helps to confirm that it is not only a product of oxidation of the tissues. (2, p. 284.)

As far as microscopical examination is concerned it is not to be preferred to the method in which chlorine is employed, as the colour is so much darker, rendering the sections more opaque, although treatment with hot water produces greater transparency and often removes any brownish shade which may mask the red colour.

The investigation was carried out in the laboratories of Birkbeck College, University of London, and has been assisted by grants from the Royal Society and the Dixon Fund of the University of London.

The author desires to express her thanks to Professor Dame Helen Gwynne-Vaughan for valuable help and advice during the progress of the work.

SUMMARY.

1. Lignified plant tissues, if treated with bromine and subsequently with solutions of sodium hydrogen phosphate and potassium iodide, give a dark red colour where the same material produces a pink colour when chlorine is used, hence a compound with bromine, analogous to that produced with chlorine, is probably formed. Thus confirmation of the association of this coloration with the presence of the halogen is afforded.

2. Washing with hot water makes the plant sections more transparent, and removes undesirable products in the cell-wall and cell-contents.

3. The cortical tissues of *L. europaea* and *Bambusa* sp., after treatment with bromine, develop a bluish coloration in sodium hydrogen phosphate solution.

4. Treatment with bromine, although producing analogous results to those obtained with chlorine, is not to be preferred.

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Studies of the Physiological Importance of the Mineral Elements in Plants.

II. Potassium: its Distribution, Movement, and Relation to Growth in the Potato.

BY

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With six Figures in the Text.

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INTRODUCTION AND METHODS.

IN the summer of 1927 a sampling experiment was laid down at the Rothamsted Experimental Station with potatoes of the variety Arran Comrade. The arrangement consisted of twenty strips embodying two manurial treatments, but responses to the treatment difference proved to be insignificant, and need not be considered further. Every strip contained three rows of sixty plants running from end to end, and each of these rows was considered to be formed of twenty triplets, and sampling was confined to the middle plants of triplets in the middle row of each strip. In this way plants with more room to develop owing to previous sampling were avoided.

Once a week, starting from the day the potatoes were planted, twenty plants, one from each strip, were gathered for measurement. The position of the plant to be taken from each strip was previously determined by drawing numbers, corresponding with those of the triplets, from a hat. Within the strips the selection was, therefore, perfectly at random, and as far as soil differences were concerned a representative sample was obtained each week. The allotted plants were dug up, the tubers carefully collected, and the whole put into a large brown paper bag and removed to an adjacent field laboratory. Here the leaf laminae and tubers were stripped from the stems and the roots pruned away and discarded. The loss of the root system was unavoidable since the soil was a very heavy clay and the collection of a representative root sample was impossible. The laminae, stalks including petioles, new tubers and mother tubers of each plant were dried separately in zinc trays. There was thus a maximum of eighty samples on each occasion, and for drying them three large ovens working at 95–100° C. were available. Since a certain amount of crowding was unavoidable, a stream of nitrogen bubbling through concentrated sulphuric acid was passed through each oven while drying was in progress. With this arrangement a satisfactory result was achieved in 48 hours. After drying, each sample was weighed to the nearest centigram.

THE DRY WEIGHT OF THE PLANT AND ITS PARTS.

The total dry weight of the plant was obtained by summing the weights obtained for leaves, stalks, and tubers. An unavoidable error was thus introduced by the omission of the weight of the roots. The magnitude of this error was estimated on July 18, about the middle of the growth period considered, by lifting four plants with every care to collect as much of the root system as possible. All large roots and their principal branches were separated, and those remaining in the soil could have formed only a very small proportion of the total weight. The dry weights of the four root systems expressed as a percentage of the total for the plant were :

$$4.72, 2.39, 4.24, 2.28. \quad \text{Mean} = 3.41.$$

It is probable, therefore, that the recorded dry weights for the plant are not on the average more than 4 per cent. too low on this account.

The mean dry weight on each occasion for the plant and each of its parts is recorded in Table I. The season was late ; planting was not carried out until May 25, and was followed by a period of drought. The first measurable sprouts appeared after heavy rain during the week ending July 6, and had an average dry weight of 0.57 grms. Up to this date the tubers had remained surface dry in the ground, slowly losing weight owing to their respiration.

TABLE I.

Weights in grammes per plant (averages of 20 plants).

1927.	Mother Tubers.			Stalks.			Leaves.			New Tubers.			Total.		
	Fresh wt.	Dry wt.	Potas- sium.	Fresh wt.	Dry wt.	Potas- sium.	Fresh wt.	Dry wt.	Potas- sium.	Fresh wt.	Dry wt.	Potas- sium.	Fresh wt.	Dry wt.	Potas- sium.
May 25	63.22	13.49	0.1739	—	—	—	—	—	—	—	—	—	63.22	13.49	0.1739
June 1	50.55	11.00	—	—	—	—	—	—	—	—	—	—	50.55	11.00	—
" 8	47.72	10.73	0.1696	—	—	—	—	—	—	—	—	—	47.72	10.73	0.1696
" 15	51.03	12.23	—	—	—	—	—	—	—	—	—	—	51.03	12.23	—
" 22	41.50	10.09	0.1335	—	—	—	—	—	—	—	—	—	41.50	10.09	0.1335
" 29	50.92	11.15	—	—	—	—	—	—	—	—	—	—	50.92	11.15	—
July 6	51.05	8.14	0.1321	4.33	0.37	0.0143	1.41	0.20	0.0062	—	—	—	56.79	8.71	0.1526
" 13	50.60	7.68	—	5.46	0.73	—	9.88	0.71	—	—	—	—	65.94	9.12	—
" 20	59.80	5.98	0.0811	33.09	2.47	0.1786	23.63	3.14	0.1459	—	—	—	116.52	11.59	0.4056
" 27	44.81	4.10	—	68.08	5.78	—	49.94	6.73	—	6.49	1.15	—	169.32	17.76	—
Aug. 3	43.62	2.85	0.0808	123.27	11.46	0.9833	96.17	13.52	0.5240	37.97	6.79	0.1557	301.03	34.62	1.7438
" 10	42.80	2.12	—	191.28	15.63	—	150.90	19.18	—	152.22	26.15	—	537.20	63.03	—
" 17	49.60	2.54	0.1113	265.19	22.49	1.356	191.48	25.57	1.053	276.23	48.03	1.110	782.50	98.63	3.6303
" 24	35.07	1.41	—	337.01	31.81	—	228.61	31.66	—	350.86	62.53	—	951.55	127.41	—
" 31	20.20	0.88	—	317.23	29.35	1.645	171.56	23.23	0.8681	531.84	107.05	2.159	1040.83	160.51	4.6721
Sept. 7	—	—	—	—	29.89	—	—	13.50	—	—	135.29	—	—	178.68	—
" 14	—	—	—	—	24.40	1.388	—	3.16	0.1118	—	165.63	2.886	—	210.65	4.7250

The total potassium value on Sept. 14 includes 0.3362 grm. found in dead leaves which had dropped off. Dry weight = 17.46 grm.

When growth had commenced no check occurred until the week ending August 24, when an infection of *Phytophthora* appeared. The weather being then dry the disease made comparatively slow progress, but the linear fall in the weight of the laminae from that date was due to the presence of the fungus, and the results are of no interest in relation to the growth of the host. The weight of the laminae at this stage was about 23 per cent. of the total recorded weight, while the young tubers accounted for 50 per cent., and were increasing their percentage rapidly. As far as could be discovered they were not directly affected by the fungus.

RELATIVE GROWTH RATES.

From the preceding measurements the relative growth-rates for the whole plant and each of its parts were calculated according to the formula

$$R = \log_e w_2 - \log_e w_1,$$

where w_1 = the dry weight at the beginning of each week, and w_2 the weight at the finish. The quantities thus obtained represent the average values of the relative growth rate during successive weeks, and may be used for comparison with other such averages.

The drift of these quantities with time is shown by the curves of Fig. 1. For a month after the week ending July 6, when active growth began, the relative growth rate of the plants taken as a whole showed a steady increase. This was then succeeded by a somewhat slower drop back towards zero as the plant aged. In general outline this picture is similar to that given for maize by Briggs, Kidd, and West (2), but there is no temporary rise in the rate associated with the period of flowering. This may perhaps be connected with the sterility of potato flowers in cultivation.

The rates of the plant organs taken individually (Fig. 2) show similar drifts, but the initial phase is much reduced, particularly in the case of the leaves. Gregory (7) has recorded a similar phenomenon for the leaves of the cucumber. It is interesting to notice that if the weight of the mother tuber is deducted from that of the whole plant (Fig. 1) the initial phase similarly almost entirely disappears from the growth curve; in other words, the lag in reaching the maximum value is due to the diminishing weight of the mother tubers, which may be regarded solely as reservoirs of material. As they are exhausted their effect becomes less apparent, and the growth curve is then controlled by those of the shoots and new tubers. There is, however, evidence of a short lag phase in the development of the individual organs in addition, which must be entirely independent of such causes, and is probably to be ascribed to the growth of the meristems, i.e. to an increase in the number of dividing cells.

POTASSIUM ANALYSES.

These analyses were carried out by Dr. N. L. Penston, using the perchlorate method as described by Cumming and Kay (4) and Brown (3), with certain modifications. Representative samples of the dried material

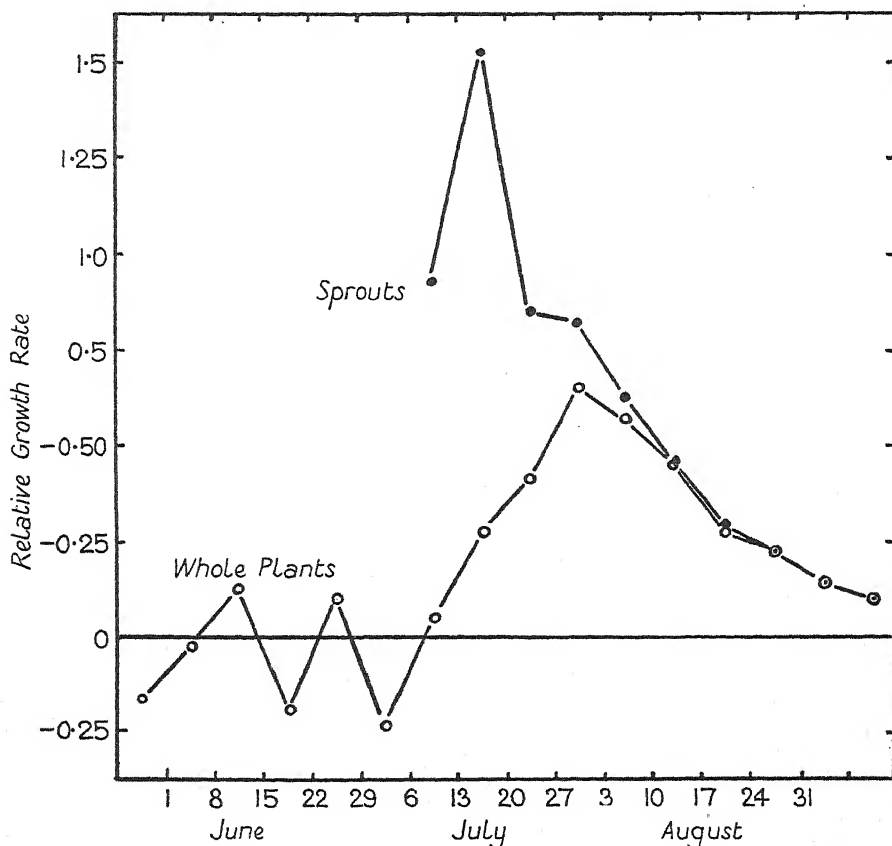


FIG. 1. Relative growth rates of the whole plant (circles) and of the sprouts = total weight less old tubers (black dots).

were obtained by grinding it to a fine powder in a mortar, and storing a sufficient amount in specimen bottles. Immediately before ashing about 2-3 grm. were redried in an oven at 100° C. for 2 to 3 hours, cooled in a desiccator, and weighed. Ashing was carried out by heating in a quartz crucible to a dull redness for about six hours and cooling in a desiccator for a standard time of 10 to 15 minutes. The ash was then weighed and analysed for potassium.

These operations were carried out on samples taken on alternate weeks, so that data are available for the potassium content of leaves, stalks,

old (mother) and new tubers, as well as for the whole plant, at fortnightly intervals throughout the life-history. The results are given in Table I (p. 427).

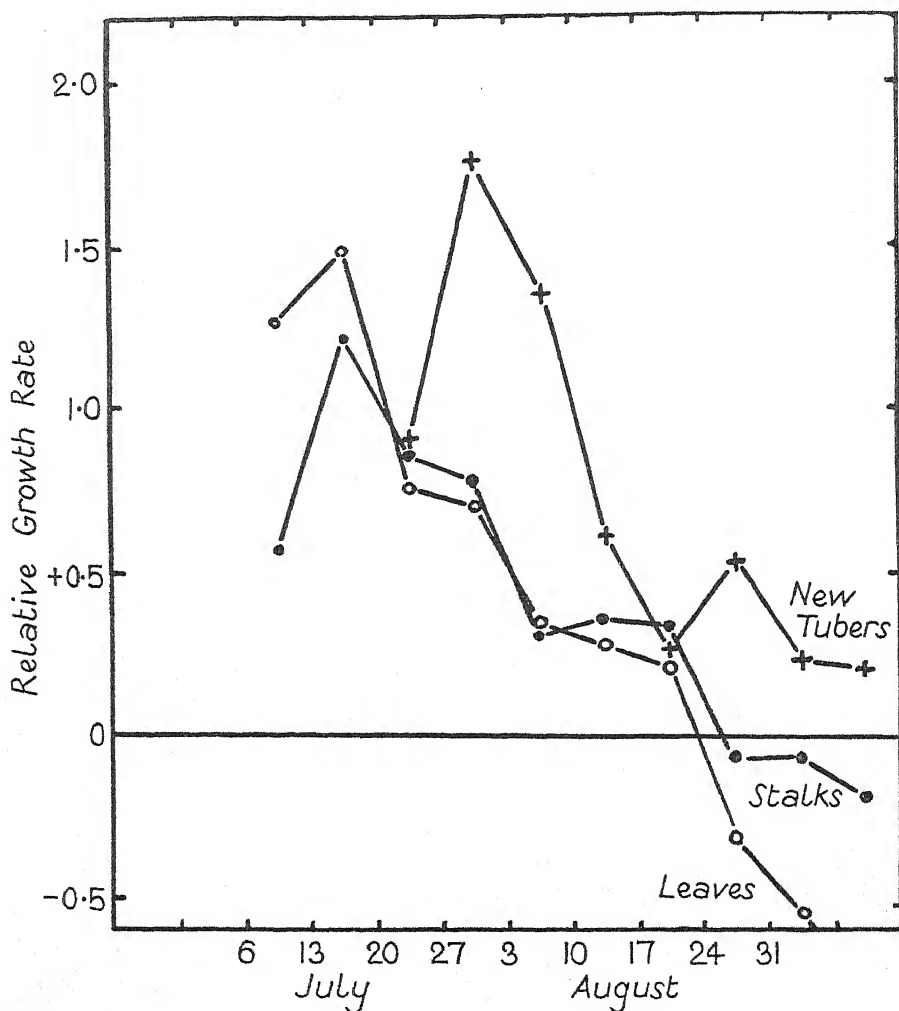


FIG. 2. Growth rates of the individual organs, leaves (circles), stalks (black dots), and daughter tubers (crosses). The negative rate shown towards the end by the leaves is due to the dropping away of the older leaves, partly as a result of the infection by *Phytophthora*.

POTASSIUM CONTENT AND RATE OF GROWTH.

In order to compare the growth rates described above with the data for potassium content it was necessary to throw them into a slightly different form. The potassium figures may be regarded as giving the mean content for the fortnight beginning one week before they were taken and

ending one week after. The corresponding growth rate is obtained from the formula

$$R = \frac{\log_e w_2 - \log_e w_1}{t_2 - t_1},$$

where w_1 is the dry weight one week before the potassium sample was taken and w_2 the weight a week after. The difference $t_2 - t_1 = 2$, the conventional period being a week.

The question arises as to the proper form in which to express potassium content. Since relative growth rate is defined as the weekly increase of a unit amount of dry weight it is clear that in this case the correct quantity to take is the amount of potassium present per unit of dry weight. It is a matter of experience that the dry weight basis of comparison is often unreliable in studies of mineral nutrition, each case requiring to be considered on its merits, and in the present paper different expressions for 'potassium content' will be used where they are more suitable. In the present instance the dry weight unit is a correct one.

The time curves of the two quantities, relative growth rate and potassium content, through the life-cycle are shown in Fig. 3. The general parallelism between the two is obvious, and a formal analysis gives a correlation coefficient of 0.9448. Even when the small number of observations, viz. eight pairs, is taken into account the probability of such a result arising by chance is much less than one in a hundred. (Fisher's Tables, 6.)

The available data allow similar comparisons to be made for each organ of the plant individually, and the corresponding curves (Fig. 4) show rather unexpectedly that there is now little connexion between the two quantities. In the case of leaves, the correlation coefficient drops to 0.1571, whereas since there are here only six pairs of observations a value of not less than 0.8114 would be required for the conventional level of significance. This immense drop cannot be explained by the reduction in the number of observations of the individual organs, but is due to the negative rates introduced by the loss in weight of stems and leaves in later stages. The lost leaf weight is accounted for mainly by the dropping off of old leaves from the lower parts of the stalks, largely owing to the fungal invasion. There may also be reabsorption of material into the stems, and the loss in weight of the latter may be ascribed to the passage of material into the still growing tubers.

As regards the whole plant it can be seen that there is a close connexion between growth rate and potassium content in the early stages. The lag in growth which occurs at this time has been shown to be due mainly to the steadily decreasing weight of the mother tubers. The simultaneous lag in potassium content is introduced by the same cause, since it is also much curtailed in the young sprouts (see Figs. 1 and 3). In stems and leaves also, despite the lack of correlation over the growth

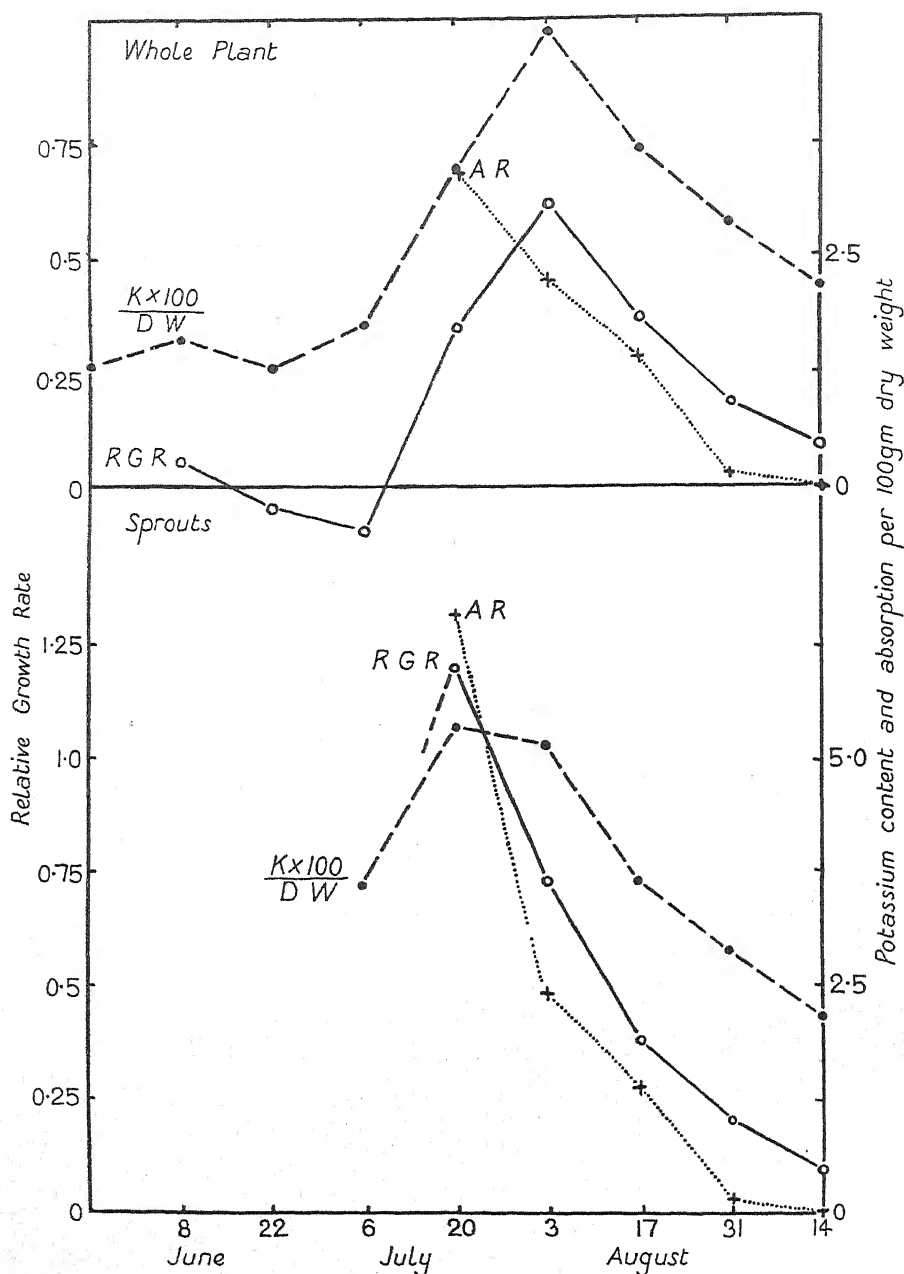


FIG. 3. Relative growth rate (RGR), potassium content ($\frac{K \times 100}{DW}$), and rate of uptake of potassium (AR) of the whole plant and the sprouts. The rate of uptake is expressed as the amount of potassium taken up per week per 100 gm. dry weight. To make it comparable with the other two quantities it is calculated in each case from values for the potassium content a week before and a week after the date given. These values were obtained by interpolation in the curve of total potassium content which is very regular.

period as a whole, both quantities again take appreciable time to rise to their highest values (cf. Figs. 2 and 4). If it were possible to obtain more frequent observations at, say, daily intervals, it might be possible to decide to what extent the two lags coincided. Unfortunately so variable a plant as the potato holds out little prospect of success in such an attempt. That a real connexion exists in the very early stages of development seems clear, however, and is highly suggestive.

In the later stages the growth rate (RGR) declines more rapidly than potassium content ($\frac{K \times 100}{DW}$), but less rapidly than the rate of absorption of potassium per unit of dry weight (AR). This is convincingly shown by the following ratios for the whole plant without the mother tubers. (See also Fig. 3.)

	July 20.	Aug. 3.	Aug. 17.	Aug. 31.	Sep. 14.
$\frac{K \times 100}{DW} / RGR$	4.49	7.04	9.69	14.64	23.50
AR / RGR	5.53	3.28	3.57	0.80	0.00

This contrast suggests that potassium is eventually heaped up considerably in excess of the amount which is necessary to maintain the declining rate of growth, and that although there may be a close connexion in the earlier stages, and although increased supplies of potassium may delay senescence for a while (James, 9), other factors control the final slowing down of growth and potassium absorption. The mechanism of the heaping up is dealt with in the next section.

It is interesting to note that potassium content when referred to the amount of water present gives essentially the same relation with relative growth rates as when referred to units of dry weight. There is one important exception to this which is afforded by the 'seed' tubers. The total potassium content of these slowly decreases owing to absorption into the actively growing organs, whereas their water content increases somewhat, presumably due to the increasing degradation of starch into sugars.

The ratio $\frac{\text{potassium} \times 100}{\text{water}}$ as a result decreases. The corresponding ratio

$\frac{\text{potassium} \times 100}{\text{dry weight}}$ increases on the other hand, showing that other solids

are passing out of the tubers relatively more rapidly than potassium, although it is usually a very mobile element inside plants (Schroeder (see 5), Rippel (11)).

POTASSIUM AND WATER CONTENTS.

From the data of Table I, the water content of the plant and its various parts can be calculated week by week. If this is expressed as quantity of water per gram of dry matter direct comparisons can be made with the

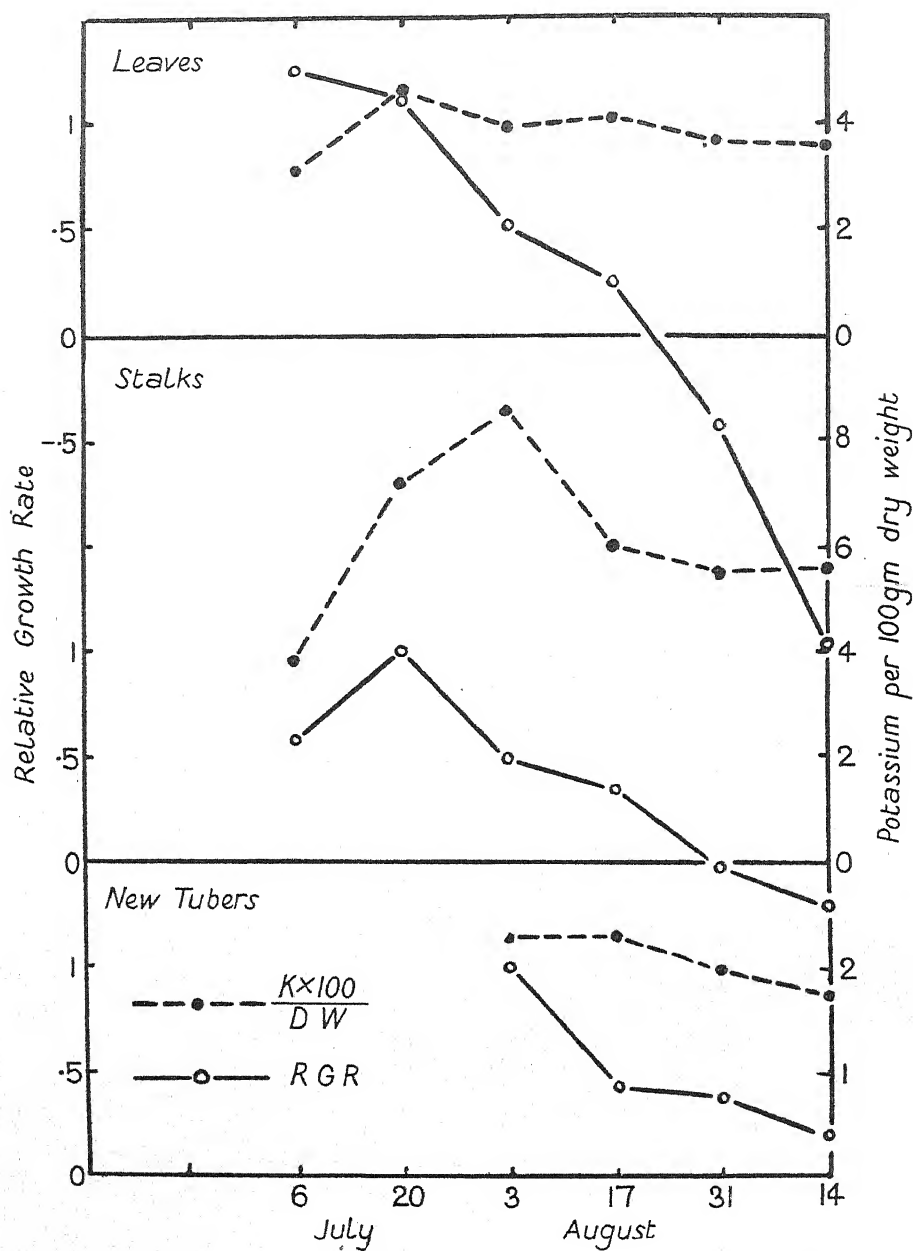


FIG. 4. Relative growth rate (RGR) continuous line and potassium content ($\frac{K \times 100}{DW}$) of leaves, stalks, and new tubers broken line.

potassium content as given in the preceding section. Fig. 5 shows how close is the relation between these two quantities. To keep the figures of convenient magnitudes water content is expressed as a direct ratio, whereas the potassium ratio has been multiplied by a hundred and given in the form of a percentage.

The correlation coefficients (r) and the probabilities of their arising by chance (P) are as follows:—

TABLE II.

	r .	n .	P .
Whole plant	0.8536	8	<0.01
Leaves	0.8116	5	0.1-0.05
Stalks	0.1	5	1

In the plant considered as a whole the correlation is thus of an undoubted significance and worthy of further investigation. In the leaves the correlation indicated is scarcely less, but owing to the smaller number of comparisons available (n) the odds against its being fortuitous have dropped to little more than ten to one. It is still high enough when considered in the light of the other data to be highly suggestive. In the stalks the correlation disappears completely, but an inspection of Fig. 5 shows that this is due to the single pair of values for August 3, and no conclusion can be based on such slender evidence.

The nature of the relationship.

In addition to the relationship at different periods of growth, potassium and water content also show similar spatial distributions within the plant. Thus both are most plentiful (per unit dry weight) in the stems, and show decreasing concentrations in the order stems > leaves > tubers.

It has been observed in some cases that an increased supply of potassium salts has led to a higher water content, and it has been concluded that the cause lies in the greater osmotic pressures associated with the additional potassium. In the foregoing analyses potassium in active parts of the plant varies between 0.5 per cent. and 1 per cent. of the water simultaneously present. (See Table III.) Assuming it to be all osmotically active and linked with a completely dissociated anion they would together give a maximum osmotic pressure of 7-14 atmospheres in the presence of a semipermeable membrane. Although this maximum value would never be realized, the two ions together might evidently contribute a large proportion of the osmotic pressures commonly found in plant cells (5-20 atmospheres; Atkins 1), and might, therefore, have an appreciable effect upon the distribution of water in the plant.

Nevertheless it seems unwise to assume that this is the only or even the principal mechanism at work. It has already been shown (James, 9)

that additional potassium, in the absence of chloride ions, has no effect upon the water content of leaves of the species under review, and results given by Gregory and Richards (8) for barley even show a decreased water content associated with additional potassium at moderate concentrations.

Since potassium is readily soluble, it is clear that the presence of water may be a cause as well as an effect of the presence of potassium ions, and a truer picture is to be obtained by considering both effects. The close numerical relationship between the concentrations of the two substances is, that is to say, the expression of an equilibrium between them, not of the fluctuations of a dependent and an independent variable. There appears in fact to be an equilibrium lying between 0.5 and 1 per cent. potassium, so that an increase in the amount of potassium above such a value must result in the retention of further water and vice versa.

It is not necessary to assume that no other causes than osmotic pressure and solution tension are at work, others, such as adsorption, are almost certain to affect the final equilibrium. If this were not so the ratio $\frac{\text{potassium content}}{\text{water content}}$ should remain constant at different times and in different parts of the plant. When, however, this value, conveniently expressed as $\frac{\text{potassium weight} \times 100}{\text{water weight}}$, is calculated, it is found to vary in a manner parallel to that of the dry weight values (Fig. 6). This is due to the fact that the total amount of water in the plant is related to the total dry weight. Since, however, there is a positive correlation between potassium content and water content per unit dry matter, there cannot also be a positive relationship between potassium and the amount of dry matter per unit weight of water, since this is the reciprocal value. In other words, the relation between potassium and water in the plant or whole organs is so important as completely to overshadow any connexion between potassium and the total solids. This does not, of course, preclude the possibility of important associations between potassium and other solids in comparatively limited positions.

THE MIGRATIONS OF POTASSIUM INSIDE THE PLANT.

Table I shows the actual amount of potassium in the various organs of the plant at the various stages of development, and from these quantities the rate of uptake and of the major movements inside the plant can be calculated. Fig. 3 gives the rate of uptake from the soil fortnight by fortnight, and, as already shown, this varies closely with the relative growth rate of the plant. The total weight of potassium inside the plant rises to the very end, and there is no evidence of any return of potassium to the soil in the later stages of growth. The distribution of this continuously

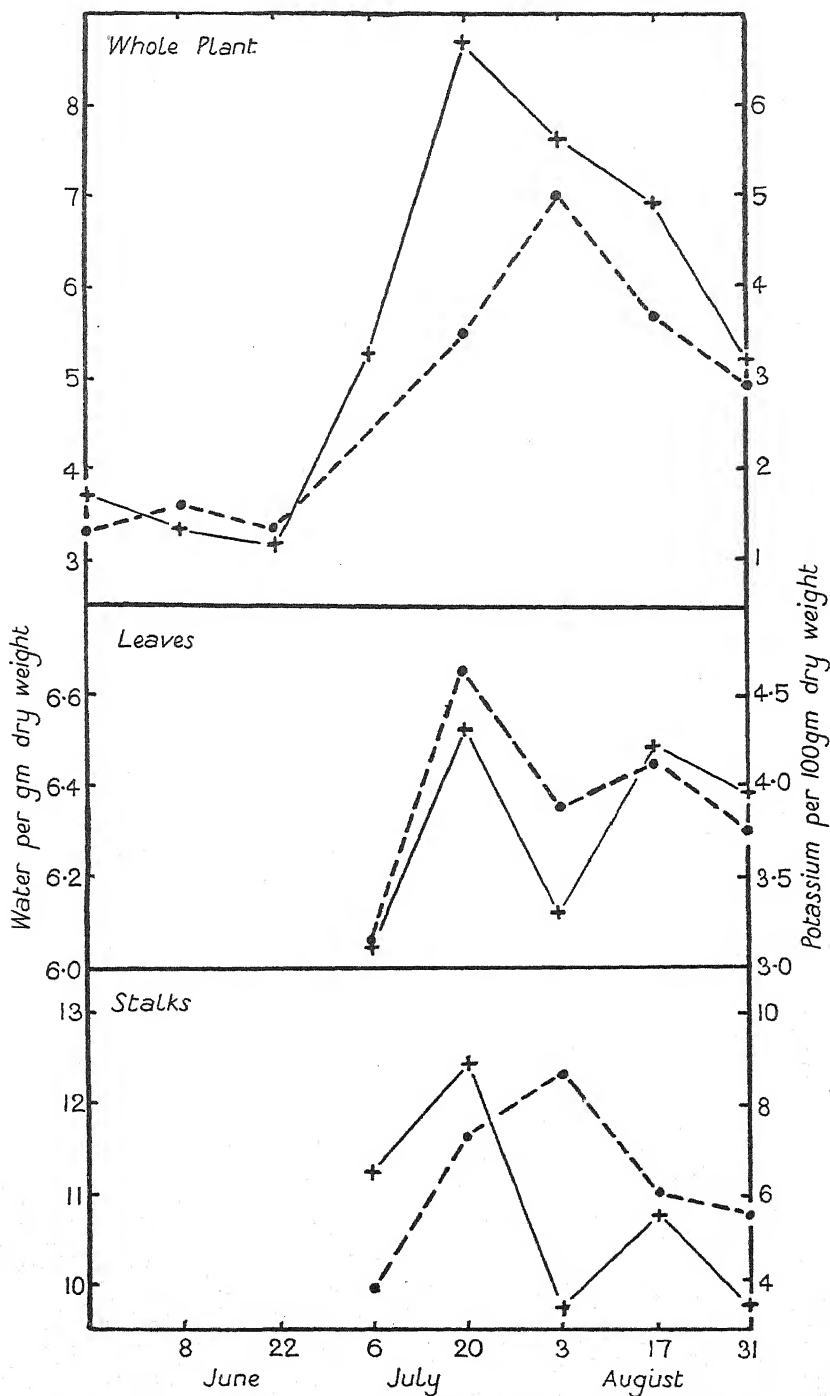


FIG. 5. Potassium content and water content of the whole plant, leaves, and stalks. Potassium content $\left(\frac{K \times 100}{DW}\right)$ is shown by the broken line, and water content $\left(\frac{\text{water}}{DW}\right)$ by the continuous line. The scales differ in the three sections of the diagram.

increasing quantity among the various organs of the plant has certain interesting features. The leaves both show a period during which they are gaining potassium to some extent from the mother tubers, but to a much larger extent from the soil. Both reach a maximum actual content, the stalks about a fortnight later than the leaves, and thereafter lose potassium to other parts of the plant, the leaves to the stalks, and the stalks in their turn to the new tubers, which increase their content rapidly at and after this time. The following migrations are thus clearly indicated :

1. Old tubers to stalks.
2. Soil to stalks.
3. Stalks to leaves. (Up to August 17.)
4. Leaves to stalks. (After August 17.)
5. Stalks to new tubers.

TABLE III.

Concentration of potassium $\left(\frac{\text{weight of potassium} \times 100}{\text{weight of water}} \right)$ *in various organs of the plant.*

	Mother Tubers.	Stalks.	Leaves.	New Tubers
May 25	0.35	—	—	—
June 8	0.47	—	—	—
„ 22	0.42	—	—	—
July 6	0.32	0.36	0.51	—
20	0.19	0.58	0.71	—
Aug. 3	0.20	0.88	0.63	0.50
„ 17	0.23	0.56	0.63	0.49
„ 31	—	0.57	0.59	0.51

When the potassium concentrations are expressed as percentages of the water present (Table III) these migrations are found to fall into two classes, those which are from a high to a low concentration and those which take place against the concentration gradient. Numbers 1-3 in the foregoing list are in the latter class, while 5 and probably also 4 belong to the former. It is significant that the movement from stalks to leaves, which may be largely due to carriage in the transpiration stream, is against the concentration gradient, whereas those in the reverse direction and into the new tubers are with it. There is thus a suggestion of a different mechanism in the downwardly directed movements, since the potassium being almost entirely water soluble (12), such 'average' gradients may be effective. Since the concentrations remain approximately constant in stems and new tubers during the fortnight August 17-31 it is possible to calculate the rate of movement during that fortnight in relation to the concentration gradient by the formula

$$R = \frac{Ql}{a(c_1 - c_2)},$$

analogous to the formula for diffusion. Expressed in grm. ions per litre,

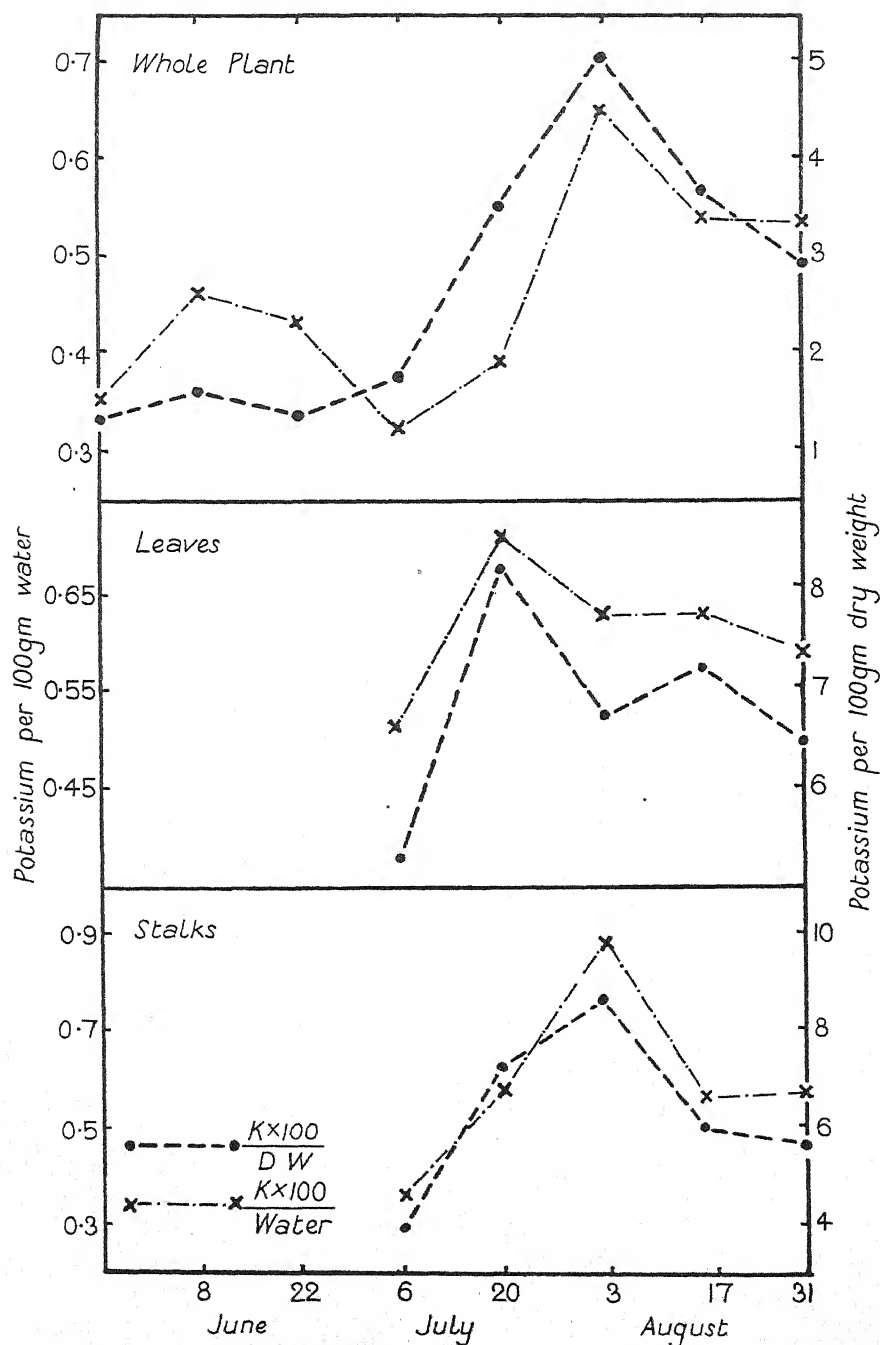


FIG. 6. Potassium content of the whole plant, leaves, and stalks expressed as percentages of dry weight and water content.

the concentration difference, $c_1 - c_2 = 0.018$; the quantity moved per second, $Q = 0.15 \times 10^{-6}$. An average value of the area of cross-section of the stalks (a) may be taken as 4 sq. cm., and the mean length of transport, (l), as 75 cm. Whence $R = 1.6 \times 10^{-4}$, or about ten times the coefficient of diffusion of potassium in water. Since it has been assumed that the whole of the cross-section of the stem is available for downward transport the value is a minimal one, and it seems certain that the actual rate must be faster than any that could result from diffusion alone.

The reversal of the movement towards the leaves is clearly indicated in Table I, and is in agreement with much other unpublished material. It has also been independently observed in other species (Czapek 5).

It seems highly improbable that a general movement towards the leaves should suddenly cease and be as suddenly replaced by a general movement in the opposite direction. Transpiration does not come to a standstill in ageing leaves, although it usually slows down. The simplest explanation of the facts is to suppose that movement is going on in both directions from the earliest stages, and that as the leaves age the movement towards them slows down, in association, perhaps, with the reduced rate of transpiration, and the return movement becoming more prominent then reduces the amount of potassium lying in the leaves. Mason and Maskell (10), working on the cotton plant, found evidence from ringing experiments for the downward movement of potassium in the stem phloem, and arrived at the same conception of a continuous circulation of inorganic ions. It is noteworthy in the foregoing results that wherever movement occurs against the concentration gradient it is in the normal direction of water movement; where it is with the gradient it is in the contrary direction. In the latter case it may be supposed that the potassium is removed from the influence of the transpiration stream, and hence perhaps that it is passing along the phloem.

SUMMARY.

1. The fresh weight, dry weight, and weight of potassium were determined at regular intervals in the leaves, stems, and tubers of potato plants throughout the season of growth. From these data, relative growth rates, potassium and water contents, and rates of absorption and migration of potassium are calculated.

2. The relative growth rate of the whole plant is similar to those already established for maize and other plants. The curves of the individual organs show a much shorter initial lag phase than that of the whole plant. The latter is shown to be due mainly, though not entirely, to the diminishing weight of the mother tubers. The residual lag may represent the growth of the meristem, i.e. increase in the number of dividing cells.

3. The time curve of potassium content of the whole plant (expressed either as percentage of dry weight or of water content) shows a significant connexion with the relative growth-rate curve ($r = 0.9448$, $P = < 0.01$). This connexion disappears in the individual organs owing to the comparatively short periods of rising relative growth rate. In later phases growth rate declines more rapidly than potassium content, but less rapidly than potassium absorption, suggesting a heaping up of potassium in non-meristematic regions (cf. 4).

4. There is a significant positive correlation ($P < 0.01$) between potassium and water contents through the growth period and a definite connexion in spatial distribution. It is suggested that the mechanism of the relation is complex, involving at least solution tension, osmotic pressure, and adsorption forces. The result is a tendency to establish an equilibrium between potassium and water at somewhere between 1 and 0.5 per cent. potassium, in the active parts of the plant, and somewhat lower in the mature tubers.

5. It is shown that potassium ions may move from one organ to another either with or against the average concentration gradient between them. The movements contrary to such gradients are always in the normal direction of the transpiration stream, while those with the gradients are against the stream.

These movements, together with the heaping up and final removal of potassium from the leaves, suggest that a continuous circulation of the element goes on. The upward movement probably depends mainly on mass movement in the transpiration stream, while the downward movement is due to some different mechanism, dependent upon concentration gradients, but faster than diffusion.

The fresh and dry weight data presented above were collected while the author was on the staff of the Research Institute of Plant Physiology of the Imperial College of Science and Technology. Their collection was only made possible by the kind assistance of Dr. F. G. Gregory and Mr. T. N. Hoblyn. The potassium estimations were carried out by Dr. N. L. Penston, and to all of these the author tenders his grateful thanks.

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Triodia decumbens, Beauv. (*Sieglingia decumbens*, Bernh.).

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With Plate XVI and four Figures in the Text.

I. INTRODUCTION.

THE seeding habits of various grasses have been under investigation for some time at the Welsh Plant Breeding Station, Aberystwyth. During the course of these studies it was observed that the florets of *Triodia decumbens* apparently did not open at all. The dissection of florets, from spikelets of what would usually have been considered but young stages in panicle exsertion, showed obvious caryopses. The examination of panicles just beginning to emerge from their sheaths showed that only the younger florets of the lowermost spikelets were without caryopses. Such florets each possessed three minute, two-lobed anthers, which were of much smaller size than the ovary. That these anthers were more or less mature was inferred from their situation over the apex of the ovary, and from the size of the latter in but slightly older flowers.

The floras and books of reference available (1, 4, 8, 9, 11, 16, 18, 21, 22, 24, 25, 27) provided no information as to the manner in which the flowers of *T. decumbens* were pollinated; those that were illustrated showed the inflorescences with linear exserted anthers. In the cases where single florets were portrayed the anthers in them were of considerable size and of the type generally met with in the grasses—that is, they were longer than broad.

The confusion caused by this situation was lessened considerably when Körnicke's paper 'Ueber autogenetische und heterogenetische Befruchtung bei den Pflanzen' came into the writer's hands.

II. AERIAL INFLORESCENCES.

Körnicke (14) states that Mertens and Koch (19) regarded the panicles of *Sieglingia decumbens* as being open flowered, 'the stigma emerging at the side of the floret', and that they are portrayed in this condition by Leers (15)

and Nees (20). This mode of flowering was contrary to Körnicke's own experience, for he had always found the florets to be 'doubly cleistogamous', the pollination occurring within the closed pales while they are still enveloped by the sheath. He therefore makes the suggestion that there must be two forms of panicle in *S. decumbens*.

In 1902 Hackel (10) published his paper 'Über das Blühen von *T. decumbens*', in which he enters fully into the whole question. He reports having received specimens with 'outstanding panicle branches and spikelet stems'. This type, he found, 'was not a separate morphological variety, but a biological form, a chasmogamous form of *T. decumbens*, which he had been seeking'. This confirmed Körnicke's suggestion. Hackel also found it to be supported by Ascherson and Graebner (2), Reichenbach¹ (23), and Beck (3), the two latter illustrating the open flowering form. The most conclusive evidence so far met with is that of Vierhapper (26), who, in 1903, observed 'on a grassy plot in the neighbourhood of Neuwaldegg, near Vienna, some chasmogamous individuals of *S. decumbens* among many cleistogamous ones. They corresponded in general characteristics to the plants described by Hackel'. On page 275 this author, referring to these same plants, states that they 'had relatively good pollen'.

Regarding the cleistogamous form, Körnicke states that the pollen-sacs were very small and placed between the very poorly developed feathery stigmas. The length of the empty dried-up oval anthers is given by Hackel as from 0.2 to 0.3 mm.

The material under study at Aberystwyth consists, therefore, of the cleistogamous type.

III. BASAL, ENVELOPED AND 'REDUCED INFLORESCENCE'.

The examination of plants of *T. decumbens* during the autumn of 1930 brought to light an unexpected and unusual structure occurring at the base of the fertile shoots and enveloped within the old leaf-sheaths. Each of these swollen bodies contained one grain.

Chase (6) described a series of reduced panicles in *Triplasis*—an American genus placed by some botanists under *Triodia* (see Bews (5) p. 161)—the extreme case consisting merely of a single-flowered² cleistogamous spikelet.

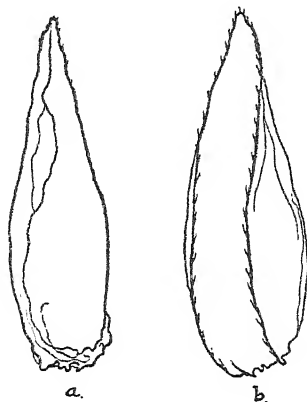
This close taxonomic relationship, and the rather striking resemblance between these basal structures, suggests that we might also be dealing with a reduced inflorescence in *T. decumbens*.

The various reduced-panicles of cleistogamous spikelets obtaining in

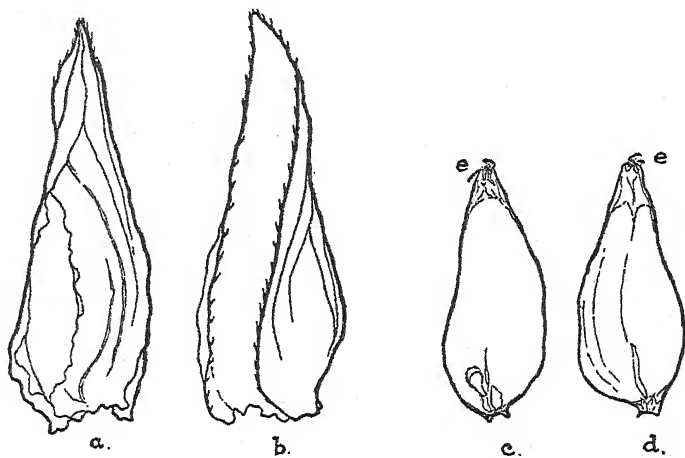
¹ According to Hackel, the description by Reichenbach is the only one which appears to tally with that of the specimens he had received.

² Sometimes a second rudimentary flower was met with.

Triplasis were described as cleistogenes by Chase. The writer, therefore, proposes to use this term, cleistogene, in order the more easily to distinguish the basal cleistogamous spikelet in *T. decumbens* from those of the aerial panicle.



TEXT-FIG. 1. Cleistogene removed from the enveloping sheaths at the base of a fertile shoot of *Triplasis decumbens*. *a*, dorsal (humped) surface; *b*, ventral (channelled) surface. $\times 7\frac{1}{2}$.



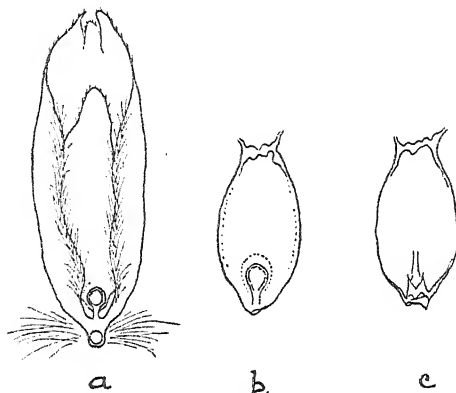
TEXT-FIG. 2. Cleistogene separated from base of fertile shoot. *a*, dorsal view; *b*, ventral view. To the right, the grain dissected from the three enveloping bracts. *c*, dorsal view showing indistinct embryo at the base; *d*, ventral view of *c*. Note remains of stigmatic hairs *e* at the apex of grain. $\times 7\frac{1}{2}$.

So far as the writer is aware, no description of the cleistogenes of *T. decumbens* has been published.

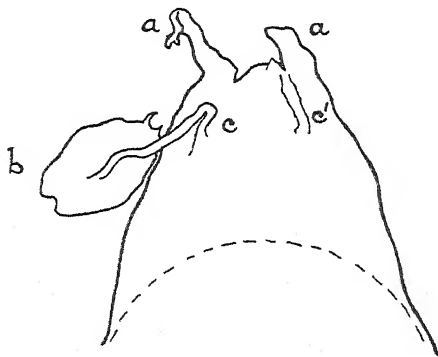
IV. CLEISTOGENES—GENERAL CONSIDERATIONS.

The cleistogenes are sessile and are found singly, attached to a basal node of the fertile shoot. Usually, especially in the earlier stages of development, they are completely enveloped by the old leaf-sheaths.

Older specimens may be seen as grey or whitish objects (Pl. XVI, Fig. 1), when plants with very ripe inflorescences are pulled apart. This would seem to be due to the splitting of the sheaths in consequence of the growth in bulk of the cleistogene within. A similar condition obtains in *Pappo-*



TEXT-FIG. 3. Mature basal floret. *a*, from aerial spikelet of *Triodia decumbens*; *b*, dorsal view of its caryopsis, showing embryo at the base; *c*, ventral view. $\times 10$.



TEXT-FIG. 4. Apical portion of grain dissected from a cleistogene. *a*, *a*, styles (stigmatic hairs were not seen in the specimen); *b*, empty, single-celled pollen-sac with filament *c*; *c'* possibly another filament. \times ca. 40.

phorum Wrightii (7), in which 'the cleistogene is sometimes so rotund as to split the sheath in which it is borne'.

The cleistogenes are different in appearance from the spikelets of the inflorescence, as may be seen from a comparison of Pl. XVI, Figs. 1 and 2.

The grain is closely encased within several, usually three, roughly pear-shaped bracts,¹ which in mature specimens are of a rather hard though somewhat brittle character.

The outermost bract is large and roughly triangular in cross-section.

¹ The number, structure, and relationship of the bracts await further investigation.

In some cases it appears to be entire, due probably to the tight overlapping of its edges to one side of the dorsal face. In fully developed cleistogenes these edges often separate slightly, as will be seen from Text-figs. 1 and 2. The ventral surface is hollowed along its vertical axis to form a U-shaped trough, which fits on to, and partially clasps, the internode above the point of its attachment (Pl. XVI, Fig. 2). The outer edges of this groove possess quite distinct upward-directed teeth, shown in Text-figs. 1 and 2. The teeth vary considerably in length as well as in number.

The second bract, partly uncovered by the opening edges of the outer one, appears to vary both in size and texture. Occasionally it was separated out as a comparatively small but broad tongue; in general, however, it approximates to the first in size and shape, but is without the vertical groove.

The third, and apparently the innermost, bract is slightly smaller, but otherwise very similar to the second in character; it completely encloses the grain.

The grain obtained from the cleistogene presents the same flinty outer appearance as the aerial caryopsis. It is rather larger and broader towards the base than the latter, while the position of its embryo is not so definitely marked (cf. Text-figs. 2 and 3).

The stigmas were very often missing in the mature grains dissected out, but some old stigmas were found. These consisted of a few rather indefinite hair-like growths arising from somewhat thickened styles at the apex of the grain (Text-fig. 2).

The anthers were even more difficult to obtain than were the stigmas, and it has not been possible from the available material to give their number. One anther is shown in Text-fig. 4, and this caryopsis certainly suggested the presence of a second anther-filament. This anther was roughly oval in shape and consisted of a single sac with a V-shaped indentation at its apex and two peg-like projections at the base.

V. DISCUSSION.

It will have been seen from Section I above that the form of *T. decumbens* with cleistogamous panicles is the one normally found, the chasmogamous type being quite rare. It is, therefore, somewhat strange to find in various English books on grasses that the inflorescences are depicted as having exserted linear anthers, while the panicle-branches are more or less adpressed to the rachis. This latter condition is considered to be characteristic of the cleistogamous inflorescence and not of the open-flowering form in which, according to Hackel, the branches spread to form an angle of 60° or more.

In view of these seeming discrepancies it will be of interest and value to know whether plants with spreading panicles and linear anthers occur in Great Britain. If the two forms are found,¹ the relative frequency of the two should be noted as well as the possibility of basal or other cleistogenes in the chasmogamous plants.

The presence of cleistogenes at the base of cleistogamous panicles was of constant occurrence in the material under test, as well as in 'wild' material subsequently collected in the neighbourhood of Aberystwyth. Since the plants were derived from several widely separated areas in West Wales, it was thought that it might represent a constant feature in *T. decumbens*. This view received strong support when plants obtained from Argyllshire, Scotland, were also found to possess these structures in abundance. It is now, therefore, confidently anticipated that these cleistogenes will be found generally present in this species.

It is interesting to note that, unlike *Triplasis purpurea*, the cleistogenes of *T. decumbens* are restricted to the base, none having as yet been found within the sheaths of higher nodes. As *T. decumbens* does not disjoin at the nodes it may perhaps be reasonably inferred that cleistogenes will only occur at the base.

The question of the viability of cleistogamous caryopses has received little or no attention. The only information so far met with is that furnished by Weatherwax (28), who found that the seeds from the aerial panicle and axillary cleistogenes 'germinate alike, and seedling plants observed until flowering, the second season after germination, are alike in appearance and vigour'. In *T. decumbens* also both kinds of caryopses have been germinated; the cleistogenes tested, however, did so only when de-husked. It is too early to make any comparisons between the seedlings from the two sources.

Chase (7) discussing *Danthonia* and other genera with their cleistogenes permanently enclosed within the sheaths surmises that they germinate inside them. This possibility was kept in mind when examining tufts of *T. decumbens*, but no evidence was found in support of this theory. The chances would seem to be against germination *in situ* for *Triodia*. The cleistogenes were often but loosely held by old sheaths—probably of a previous season's growth—and they have, in fact, been found free when old plants were being broken down.

The observations recorded in this paper must necessarily be incomplete, owing to the unsatisfactory nature of mature specimens as the medium for investigation. Many interesting features have, however, presented themselves, and it is proposed to throw more light on them during 1931, when the development of the cleistogenes will be followed in living material.

¹ The writer would welcome specimens or the chasmogamous panicles, and would be especially grateful for living tillers of such plants.

VI. A SUPPOSED HYBRID DERIVATIVE OF *SIEGLINGIA DECUMBENS*.

It was considered by the writer that a paper dealing with *T. decumbens* would be incomplete without some reference to *Danthonia breviaristata*, Beck. Vierhapper in his interesting paper (26) discusses at length his contention that *D. breviaristata* is really a hybrid between *S. decumbens* (L.), Bernh. and *D. calycina*, Vill., and not a variety of the latter. The hybrid is found in two forms, 'chiefly cleistogamous, but chasmogamous individuals are not too rare'; and he states that he saw 'both forms in fairly large number growing with the parents'.

The truth of Vierhapper's conclusion can be proved beyond doubt only by the artificial reproduction of *D. breviaristata* under controlled conditions (12) from the rare chasmogamous *S. decumbens* and *D. calycina*. This cross, if successfully achieved, might throw light upon the inheritance of the phenomena of cleistogamy and chasmogamy, as well as upon the taxonomic relationship of these two genera. Whether its production should imply the removal of *Sieglingia*¹ to *Danthonia* is problematical, for Jenkin (13) in his work on inter-specific and inter-generic crosses has proved that the latter crosses may, in certain cases, be more readily accomplished than the former.²

VII. SUMMARY.

1. *T. decumbens* possesses aerial cleistogamous panicles, the florets of which are fertilized while still enclosed within the sheath.
2. A rare form with chasmogamous aerial panicles is known, but this was not met with among the material under study.
3. Single-flowered, close-fertilized spikelets—'cleistogenes'—are recorded for the first time in this species.
4. The cleistogenes are sessile, and arise singly from a basal node of the fertile tiller. They are usually hidden by the leaf-sheaths enveloping the flowering stem.
5. The caryopses from the aerial panicle as well as those from the cleistogenes are viable.
6. *D. breviaristata*, Beck., considered by Vierhapper to be a hybrid between *S. decumbens*, Bernh. (*T. decumbens*, Beauv.) and *D. calycina*, Vill., is briefly commented upon.

The writer wishes to express his thanks to Professor R. G. Stapledon, M.A., Director, Welsh Plant Breeding Station, for providing the necessary

¹ *Sieglingia* is in fact placed under *Danthonia* by Stapf (see Hubbard in Appendix to Bews (27), p. 385).

² Mallock (28) inter-crossed *Hordeum vulgare* and *H. murinum*, but he obtained two seedlings only, and these died when a few inches high.

facilities and for his interest in the work. Thanks are also due to the following:—Mr. T. J. Jenkin, M.Sc., for helpful criticism; Mr. D. W. Davies, B.Sc., for taking the photographs; Mr. M. T. Thomas, B.Sc., for collecting material in Argyllshire; Miss Rhoda Jones, B.A., and Miss G. M. Roseveare for assistance with the literature.

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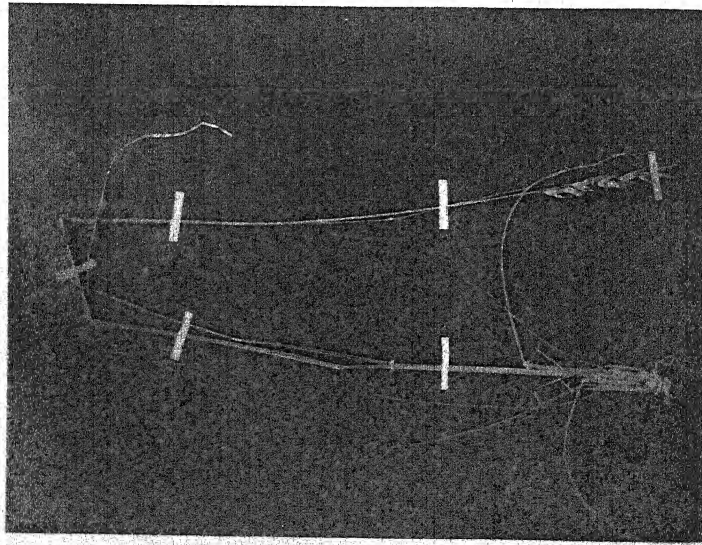
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EXPLANATION OF PLATE XVI.

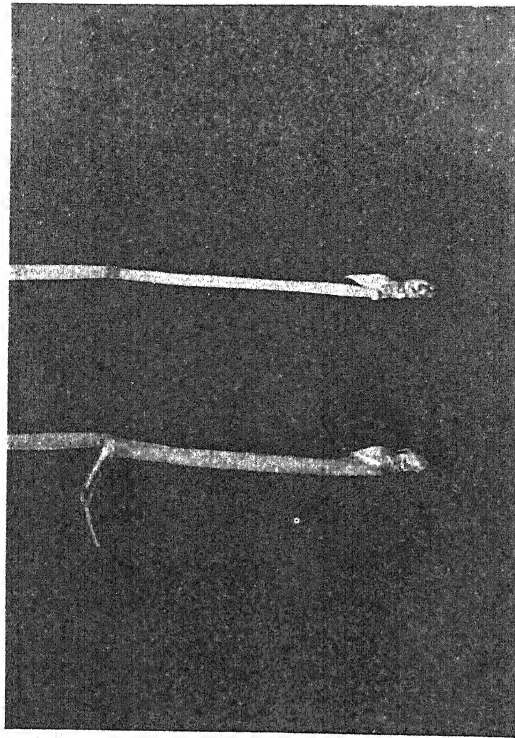
Illustrating Mr. A. R. Beddows' paper on *Triodia decumbens*, Beauv.
(*Sieglingia decumbens*, Bernh.).

Fig. 1. A panicle shoot of *Triodia decumbens* with cleistogene (*c*) at its base. The sheaths, here were partly removed to bring the cleistogene into view. ($\times \frac{1}{3}$.)

Fig. 2. The basal portion of two panicle tillers of *Triodia decumbens*, with sheaths removed to show the cleistogenes at their base. The ventral (grooved) side is seen to lie close against and partially clasp, the stem (nat. size).



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Huth coll.

BEDDOWS — TRIODIA DECUMBENS.

The Horizon of '*Lepidodendron harcourti*'.

BY

R. CROOKALL, PH.D.

With Plate XVII.

I. INTRODUCTION.

IN 1831, Witham of Lartington figured and described the first discovered specimen of *Lepidodendron* with both external and structural preservation, naming it *L. harcourti*. Further examples of the species were figured by Lindley and Hutton, and by Brongniart, while more recent and detailed researches have been due to C. E. Bertrand (2) and others. The original stems have always been regarded as coming from Lower Carboniferous rocks, though the many examples of *L. harcourti* which have been subsequently found are known to be of Upper Carboniferous age.

In 1887, the late Professor W. C. Williamson (12) transferred some of the Upper Carboniferous specimens which he had previously referred to Witham's species to *L. fuliginosum*, and later (13), pointing out that Witham's and Lindley and Hutton's descriptions contained many errors, amplified the account of the species on the basis of specimens collected by Mr. J. Lomax from the Lower Coal Measures of Dulesgate, Lancashire. In 1907, however, Professor D. M. S. Watson (11), showed that Williamson's Dulesgate specimens belonged to a distinct plant, which he named *L. hickii*. Apart from these forms a number of stems which must be identified with Witham's *L. harcourti* are known from several Upper Carboniferous localities, including Shore, Oldham, Stalybridge, Dulesgate, and Halifax.

In 1903, Dr. Kidston (5), in briefly reviewing the species, was exercised by its apparent occurrence in both the Lower and Upper Carboniferous, observing that, 'not a single species of *Lepidodendron* which occurs in the Lower Carboniferous has been known to pass into the Upper Carboniferous.'¹ He was, however, unable to state any specific differences between

¹ Kidston must be regarded as here referring to petrifications only. In 1906, dealing with impressions, Kidston (7) pointed out that 'only one Lower Carboniferous species, *Lepidodendron veltheimianum*, Sternb., passes into the Upper Carboniferous, and this only extends into the Millstone Grit, not passing so far up as the Slatyband.' This statement was based on a more detailed account published in 1910 (6), where not only *L. veltheimianum* but also *L. glincanum* (Eichwald) were recorded from supposed Upper Carboniferous rocks. Kidston subsequently suppressed the latter record (no doubt questioning his determination) but repeated the former. Mr. C. H. Dinham (*in litt.*) has pointed out, however, that the evidence for the Upper Carboniferous age of the deposits is more than doubtful.

the types and the Coal Measure examples. A comparison of the known types (Pl. XVII, Figs. 1, 2) with Upper Carboniferous specimens (Pl. XVII, Figs. 3, 4) reveals no important point of difference.

II. HISTORY OF THE TYPES

The history of the types is in a very unsatisfactory condition.

In 1831, H. Witham (14) described the first specimens and, though he did not mention their locality and horizon, on the glass of the (original) preserved section, in the York Museum, is written, 'Northumberland Limestone, Vernon'—see Pl. XVII, Fig. 1. It also bears a label, written probably by Williamson, giving 'Hesley Heath' as the locality, and claiming that the slide was figured by Lindley and Hutton (Pl. XCVIII) and, on another part, 'figd. Lindley and Hutton, Pl. XCIX.' There is another label, in Kidston's handwriting and initialed by him, as follows, 'Specimen figured by Witham in Trans. Hist. Soc. of Northumberland, Durham and Newcastle-on-Tyne, Vol. II, Pl. II, Fig. I, 1838 (paper read March 1832)—also in "Internal Structure of Fossil Vegetables", Pl. XIII, Fig. 2, 1833' (and a pencil correction to Fig. 1).

The specimen, as drawn by Lindley and Hutton (Pl. XCVIII, Fig. 1), is longer than that in the York Museum (but this may be accounted for by the fact that both Williamson and Kidston had it cut for purposes of comparison). If Lindley and Hutton's figure can be relied upon, their specimen was 6.4 cm. long. In width the two agree. But the (somewhat decorticated) leaf-bases, as drawn by Lindley and Hutton, are markedly further apart than those on the York specimen—in the vertical direction only. Laterally they are about the same distance apart, about 6 mm. Comparing this section with Lindley and Hutton's Pls. XCVIII, XCIX, the two cannot be matched. In Pl. XCIX, Fig. 1, the leaf-traces are accompanied by a 'colourless ring' (disintegrated tissue) such as is not found in the section. This, and other features, show that Kidston was justified in his statement that the York section was not made from Lindley and Hutton's type. On the other hand, it corresponds with Witham's Pl. V, Fig. 1 (1832),¹ and Pl. XIII, Fig. 1 (1833), as shown by Kidston and confirmed by Dr. Scott. Through the kindness of Dr. Walter E. Collinge, I have been able to compare the section with Witham's figures, and to confirm the identification with Witham's Pl. V, Fig. 1.² The Annual Report of the York Museum for 1832 contains, among the 'Donations to the Museum' the following, 'Rev. C. V. Harcourt... the specimen of *L. harcourtii*

¹ Kidston (5) cited Plate II, Fig. 1 in error.

² A further section (No. V. 1829) occurs in the General Collection of the British Museum (Natural History), and a pencil note by the late Dr. Arber suggests that it is 'part of Lindley and Hutton's specimen from the type at York'. As will be seen, however, Lindley and Hutton's specimens were considered to be at York in error. As in the case of the York section, there is written on the glass, 'Northumberland Limestone, Vernon'.

from which Mr. Witham's section was taken.' The reference of this specimen to 'Hesley Heath' is evidently correlated with the erroneous identification with Lindley and Hutton's specimen.

In 1833, Witham (15) described and figured the same specimen, and gave the same figure on Pl. XIII at Fig. 1 as he previously gave on Pl. V, Fig. 1. He here (p. 51) observed that the 'specimen was obtained by the Rev. C. G. V. K. Harcourt, Rector of Rothbury, and (p. 56) gave the locality as the 'North of England'. It may be observed that the Nichol Collection (British Museum, Natural History) contains four slides of *L. harcourtii*. Of these one (V. 9327) is marked 'Rothbury, Northumberland', and is probably the same as Witham's Pl. XII, Fig. 4, while three (51883-5) are labelled 'Midlothian', although they possibly came from the same specimen. In all four cases the writing is scratched on the glass.

Lindley and Hutton (8) described as *L. harcourtii* specimens from 'a bed of coal worked at Hesley Heath, near Rothbury in Northumberland: it is there found in a few fathoms below a thick limestone which is by some considered analogous to the great limestone of Alston Moor'. This stem was either destroyed in sectioning, or has remained unrecognized (in spite of statements to the contrary). Apparently, however, other examples occurred, as Lindley and Hutton speak of 'fossils'. For many years it was supposed that one of Lindley and Hutton's specimens was in the York Museum, but, as observed above, Kidston proved this to be an error.

In 1839, A. Brongniart (3), in his account of *Sigillaria elegans*, included a figure of *L. harcourtii*, the locality of the specimen being given as 'England' (p. 407). This section was from the stem now preserved in the York Museum. The stem is nearly 4 cm. in length and oval, the greater axis measuring 4.5 and the lesser 3.6 cm. Kidston had a further section cut from this stem (also now in the York Museum—Pl. XVII, Fig. 2) and (loc. cit.) observed, 'apparently Brongniart possessed little more than a transverse section, as most of the longitudinal sections he publishes are copies of Witham's and Lindley and Hutton's figures.' Kidston's section is marked 'No. 4' and bears, in his handwriting, 'Portion of specimen given by Hutton to Brongniart, and figured by him in "Observations sur la structure..." p. 432, Pl. (VI). XXX, Fig. 5'. This, again, cannot be matched with Lindley and Hutton's figures. A section which clearly came from the same specimen is preserved in the Williamson Collection at the British Museum (Natural History, no. 1593). This bears, in Williamson's handwriting, the following label, 'Section of the original specimen of *L. harcourtii* in the York Museum, figured by Brongniart and Lindley and Hutton'. (It is again necessary to point out that the slide was not from Lindley and Hutton's specimen.)

In 1887, Williamson (12), giving no locality, mentioned *L. harcourtii*, and (erroneously) said that Lindley and Hutton's original specimen was in the York Museum, and, in 1893 (13) repeated Lindley and Hutton's locality of Hesley Heath.

In 1903, Kidston (5) reviewed the species after examining the available specimens and slides (except, apparently, those in the British Museum), repeated Lindley and Hutton's locality of Hesley Heath, and added the horizon as 'Calciferos Sandstones'.

So far as the locality and horizon of the types are concerned, it will be seen that:

(1) *Witham's type, according to the writing on the glass, which cannot be relied upon, came from a Northumberland Limestone. The locality given in the text is merely 'North of England', and the fact added that it was obtained by the Rev. Harcourt of Rothbury. A transverse section is in the York Museum, and one in the British Museum (v. 1829.)*

(2) *Lindley and Hutton's *L. harcourtii*, different specimens, though supposed by Williamson to be preserved at York, cannot be traced. They were described as coming from Hesley Heath.*

(3) *The horizon 'Calciferos Sandstone Series' is due to Kidston, who observed that the specimens came from 'rocks belonging to the Scremerston Group of the Calciferos Sandstone Series'.*

We have now to examine the value of the evidence, both as regards locality and horizon. As is well known, some of the early fossil botanists did not realize the importance of keeping accurate records of the origin of their specimens, and even Williamson did not always pay due attention to the horizon of his plants (having described Lower Carboniferous species from Upper Carboniferous rocks). Lindley and Hutton, however, were particularly unreliable in this respect. To cite three instances only, they (9) described their *Sphenopteris hoeninghausi* (*non S. hoeninghausi*, Brongniart) = *S. effusa*, Kidston, as 'from Felling Colliery, Northumberland', while Mr. R. Howse (4) has cast grave doubts upon this origin and suggested that it came from 'the Calciferos Sandstone Series of the neighbourhood of Edinburgh'. Again, Lindley and Hutton described their *S. obovata* (8) and *S. excelsa* (9) as from the 'Newcastle Coalfield', but the former was received from Mr. T. Allan of Edinburgh, and both Mr. Howse and Dr. Kidston satisfied themselves that they were actually from the Calciferos Sandstones of the Edinburgh district.

Witham's specimen merely came from the 'North of England', while Brongniart merely gave the locality as 'England'.

With regard to Lindley and Hutton's locality of 'Hesley Heath', we have first to observe that no such town, village, or parish exists, either in Northumberland or any other part of Britain. Moreover, according to the

Rev. Dr. J. E. Hull (*in litt.*), of Belford Vicarage, Northumberland, the word 'heath' is not used in Northumberland. There is, however, a Hesleyhurst two miles S.E. of Rothbury, but no colliery has existed there, though there were old 'bell-type' workings which have long been abandoned. Although the district has been searched by my colleague Mr. G. A. Burnett, no petrified plants have been found. Again, a colliery is at present working some distance to the E. of Low Hesleyhurst,¹ drawing coal from seams in the Upper Limestone Group (not the Scremerston Measures, as given by Kidston) but this also has proved barren of petrifications. There is also a Hesleyside in the North Tyne area and this lies in the Scremerston Measures, but this is too far from Rothbury to represent the origin of Lindley and Hutton's specimens. Finally, one horizon only is known in Northumberland yielding 'coal-ball' petrifications, namely, the Little Limestone Coal, near Brandon-on-Tyne (and this, according to Absalom (1), may be tentatively correlated with the Chirm Seam). The original specimens of *L. harcourtii* were isolated and not of the 'coal-ball' type.

It will be seen that :

- (1) *There is no such locality as Hesley Heath, near Rothbury.*
- (2) *No locality near Rothbury produces or has been known to produce petrifications.*
- (3) *There is no basis for Kidston's reference of the fossils to the Scremerston Measures.*

As Harcourt was a Yorkshireman, reared at the Bishopthorpe Palace, York, it seems not unlikely that Witham's specimens actually came from the Lower Coal Measures of the Yorkshire Coalfield, having been collected either by Harcourt or by some friend who knew of his interest in geology. The plant is known from the Lower Coal Measures of Yorkshire. If, however, we are to regard 'Hesley Heath' as affording any clue to Lindley and Hutton's and to Brongniart's specimens (which is very doubtful), the only possible Yorkshire locality is Thorpe Hesley, near Rotherham. Here, according to Mr. C. N. Bromehead (*in litt.*), the Parkgate Coal and the Tankersley Ironstone were formerly almost certainly worked, but the beds have afforded no petrifications.

We may here sum up the position as follows :

There is no evidence that the types of L. harcourtii came from Northumberland. It is possible that they came from Yorkshire. No other species of Lepidodendron is known from both Lower and Upper Carboniferous rocks, and we are therefore justified in assuming that they were of Upper Carboniferous origin.

¹ Two coals have been mined in the neighbourhood of Low Hesleyhurst, three miles S.E. of Rothbury, (a) the lower coal at Lee Colliery in Group d 2b (Middle Limestone Group), (b) the higher coal, one mile E.S.E. of Low Hesleyhurst, known as the Chirm Seam in Group d 2c (Upper Limestone Group).

It is true that *L. harcourtii* has resemblances (in the projecting ridges of the xylem) to the Lower Carboniferous species *L. wünschianum* (Will.), and Professor Seward (10) has suggested that *L. harcourtii* may be only a small form of *L. wünschianum*, the latter having well developed secondary xylem. The plant shows affinities, however, with the Upper Carboniferous form *L. fuliginosum*, Will. (with which, indeed, it was originally confused by Williamson). These resemblances can have no weight in determining the horizon of the types.

III. OTHER REPUTED LOWER CARBONIFEROUS EXAMPLES.

While the Scott, Williamson, Nichol, and General Collection of slides in the British Museum (Natural History) contain examples of *L. harcourtii* from the Upper Carboniferous of Dulesgate, Oldham, Stalybridge, Halifax, &c., there is none from reputed Lower Carboniferous horizons.¹ On the other hand, the Kidston Collection of slides (in the Botanical Department, Glasgow University) contains a single slide (no. 855) identified as *L. harcourtii* and labelled 'Dalry, Ayrshire, Carboniferous Limestone Series, from D. Landsborough, L.L.D.'. Dr. S. Williams kindly took several photomicrographs of this stem and forwarded them to me for examination. There can be no doubt that it is distinct from *L. harcourtii*. The points of the protoxylem groups are not sufficiently prominent and the xylem cylinder is too thick. Dr. Scott, who examined the photographs, confirmed this conclusion.²

I have pleasure in thanking Dr. D. H. Scott for calling my attention to this problem, and for his help and encouragement in this and other investigations. Acknowledgements are also due to Dr. Walter E. Collinge for the loan of the types which are in his charge in the York Museum, to Messrs. G. A. Burnett and A. Fowler of the Geological Survey for information with regard to the Carboniferous rocks and localities in Northumberland, and to Mr. W. N. Edwards, of the British Museum (Natural History) and Dr. S. Williams, of the Department of Botany, University of Glasgow, for assistance in connexion with the sections of *Lepidodendron* in their care.

IV. CONCLUSIONS.

The following sections appear to represent portions of the types of *L. harcourtii*: (a) the original slide, York Museum; (b) Kidston's slide (No. 4), York Museum; (c) British Museum (Natural History)—(i) General

¹ Except, as mentioned above, in connexion with the types.

² Dr. Scott observed (*in litt.*) that in these two respects the stem is more like *L. brevifolium*, Will. (= *L. veltheimianum*, according to Kidston), though he has not seen a stem of *L. brevifolium* which was of this size and in which secondary thickening was absent.

Collection, No. V. 1829; (ii) Nichol Collection, probably V. 9327 and 51883-5; (iii) Williamson Collection, 1593.

Brongniart possessed a single transverse section. Lindley and Hutton's slides, which were distinct, cannot be traced.

The locality and horizon of the types are unknown. The horizon (Calcareous Sandstone Series) was determined by Kidston on the supposition that the locality of Lindley and Hutton's specimens was Hesley Heath, Northumberland. No such locality exists.

All specimens of *L. harcourti*, the origin of which is definitely known, are of Upper Carboniferous age.

Until examples of the species are obtained from Lower Carboniferous rocks, 'L. harcourti' must be regarded as being confined to the Upper Carboniferous.

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 15. ——— : The Internal Structure of Fossil Vegetables, &c. Edinburgh, pp. 51, 56, 75. Pl. XII, Figs. 1-7; Pl. XIII, Figs. 1-7, 1833.
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EXPLANATION OF PLATE XVII.

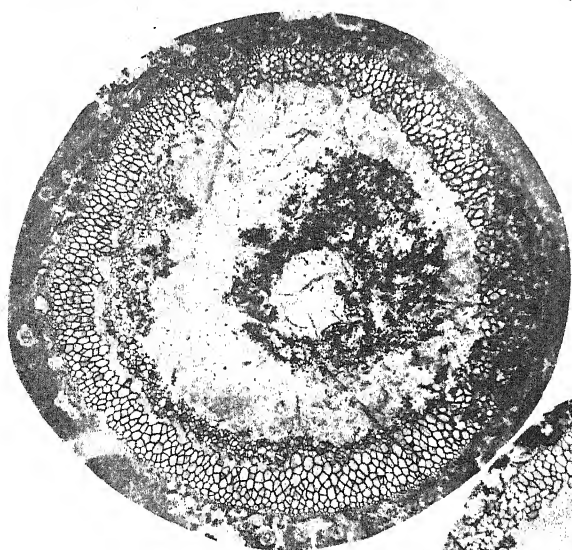
Illustrating Dr. R. Crookall's paper on the Horizon of '*Lepidodendron harcourti*'.

Fig. 1. *Lepidodendron harcourti*. Photomicrograph of section in York Museum (figured by Witham, 1832, Pl. II, Fig. 1; 1833, Pl. XIII, Fig. 1). A similar section is in the British Museum (Natural History, No. 1829). $\times 8\frac{4}{5}$.

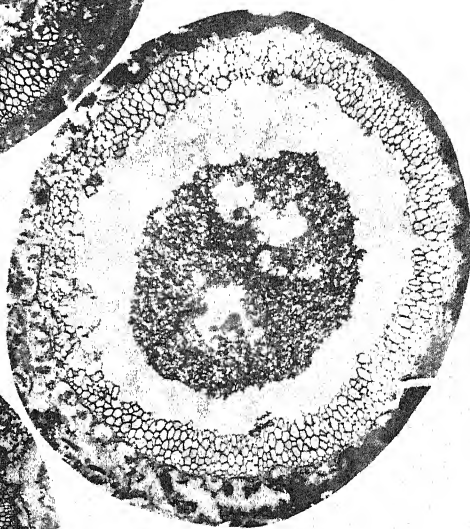
Fig. 2. *Lepidodendron harcourti*. Photomicrograph of section in York Museum (cut at Kidston's instigation) (similar to that figured by Brongniart, 1839, Pl. XXX, Fig. 5). A further section, cut by Williamson, is in the British Museum (Natural History, No. 1593). $\times 8\frac{4}{5}$.

Fig. 3. *Lepidodendron harcourti*. Specimen from Lower Coal Measures of Shore, Littleborough, Lancashire. $\times 8\frac{4}{5}$.

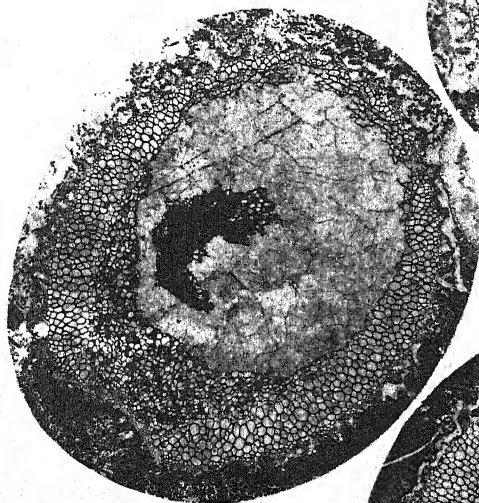
Fig. 4. *Lepidodendron harcourti*. Specimen from Upper Foot Mine, Oldham, Lancashire (Lower Coal Measures). $\times 8\frac{4}{5}$.



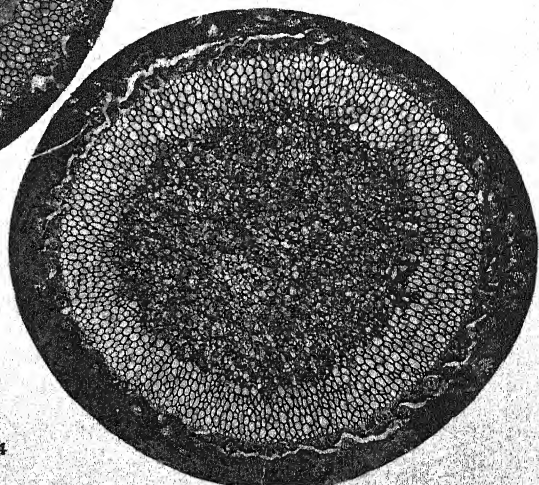
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On the Use of the Katharometer for the Measurement of Respiration.

BY

WALTER STILES

AND

WILLIAM LEACH.

With seven Figures in the Text.

INTRODUCTION.

IN plant physiology there is a very definite need for a method of measuring the respiration of plant tissue which shall fulfil certain conditions. These are that the method shall (1) be applicable to small quantities of plant material, (2) allow of a continuous record of the respiration rate, (3) permit of determination of both the carbon dioxide evolved and the oxygen absorbed, and (4) be reasonably accurate.

None of the usual methods of measuring plant respiration, although reasonably satisfactory for the work for which they have been employed, fulfil all these conditions. The only method at present available which appears to do so is that depending on the use of the instrument called by its designer the katharometer. The first attempt to utilize the principles involved in this instrument for measurement of respiration was made in 1922 by Noyons (6), who recorded a few measurements of the concentration of carbon dioxide in expired air from a rabbit. The katharometer itself appears to have been first employed for work on respiration by Slater, who described his apparatus in 1926 (8), and who published results obtained with it on the respiration of the cockroach shortly afterwards. Subsequently accounts of work involving determinations of plant photosynthesis and respiration by means of the katharometer have been published by Waller (10) and Gregory and Richards (3).

The examination of the possibility of utilizing the katharometer for the measurement of gaseous exchanges of plants was begun by one of us in the Botanical Department of the University of Reading in the autumn of 1928, and was continued by both of us in the Botanical Department of the University of Birmingham in the autumn of 1929.

In the course of this work many difficulties and sources of error were encountered, and ways of dealing with them devised, and step by step an arrangement by which plant respiration may be accurately measured by the katharometer was elaborated. As no adequate account of their apparatus has been published by other investigators, as none of these investigators describe all the difficulties and sources of error which appear as pitfalls for the unwary, as in some cases the respective experimental arrangements used by different investigators exhibit divergent behaviour, and as none of these investigators have made it clear that they were working with an apparatus that was free from all sources of error, it seems most desirable that a detailed account of this most valuable method of determining plant respiration should be put on record.

THE KATHAROMETER.

Principle of the instrument. When an electric current is passed through a wire the latter becomes heated and its resistance changes. The temperature at which the wire is maintained above that of its surroundings depends, among other factors, on the rate at which heat is removed from it, and this in turn depends upon the thermal conductivity of the medium surrounding the wire. The thermal conductivity of the medium depends upon its composition. Hence, a change in the composition of a gas surrounding a wire carrying an electric current will result in a change in the electrical resistance of the wire, and if care is taken to control other factors this change in electrical resistance can be utilized as a means of measuring the change in the composition of the gas. It is this principle of which use is made in the katharometer and similar instruments that have been devised for measuring the concentration of any particular gas in a mixture, as, for instance, the concentration of carbon dioxide in air.

The development of practicable instruments for gas analysis based on this principle took place in England, Germany, and America just before and during the Great War, with the result that, owing to absence or difficulty of intercommunication, different types of instruments were developed in complete independence of one another in the different countries. Of these different instruments the one designed in the University of Birmingham by Dr. G. A. Shakespear, and called by him the katharometer, appears to have definite advantages in design over the other types, especially in regard to portability, and appears to be the only instrument of its kind that has come into general use.

In all types of instruments the general electrical arrangements are the same, inasmuch as the heated wire forms an arm of a Wheatstone's bridge. Change in composition of the gas surrounding the wire, by altering its resistance, throws the bridge out of balance. The change in composition

of the gas is determined either from the galvanometer deflexion produced or by measuring the amount by which the resistance of another arm of the bridge must be changed in order to bring the latter into balance again. In either case calibration with gases of known composition is necessary.

An inherent limitation of the method is that it can only give results with comparatively simple gas mixtures of known constituents; it cannot be used for qualitative analysis. But for the quantitative analysis of mixtures of two gases, or of a mixture in which only two of the constituents undergo change, the method is eminently suitable.

Both the Shakespear katharometer and the other experimental arrangements based on the same principle have been described by their designers or users, so that it will only be necessary here to mention the essential features of the different types.

The Koepsel arrangement. This apparatus was designed by Koepsel for the measurement of the concentration of hydrogen in producer gas, and was improved by Siemens and Halske, and patented in Germany in 1913. Some description of this apparatus has been given by Weaver and his collaborators (11). Four similar wires constitute the arms of a Wheatstone's bridge; two opposite arms are enclosed in a chamber through which passes a stream of the gas under examination, while the other two arms are similarly enclosed in a chamber through which passes a stream of gas of standard composition. The deflexion of the galvanometer indicates directly the percentage of the constituent gas, the constitution of which is varying, and the galvanometer takes the form of an indicator calibrated in percentages of this gas. It is an essential of this, and of all other arrangements, that the heating current flowing through the bridge shall be constant. This is achieved by connecting a rheostat in series with the battery which serves as the source of current. For measuring the strength of the current supplied to the apparatus the indicating galvanometer is employed; by means of a cross-over switch it can be connected at will, either across the bridge circuit, where it records changes in the gas, or in the heating circuit. When in the latter position it is protected by means of a shunt and functions as an ammeter.

Noyons, a description of whose apparatus will be given later, states that the Siemens and Halske apparatus is less sensitive than his own. As Noyons' apparatus appears to be none too sensitive it is clear that the Siemens and Halske apparatus is unsuitable for work on respiration, for which, indeed, it was not designed.

The Shakespear instrument. The katharometer was designed by Shakespear in 1915 for purposes connected with the War, and early accounts of its applications formed the subject of Reports of the Advisory Committee on Aeronautics during the years 1916-18. An account of the theory of the instrument was published by Daynes (1) in 1920, while short

accounts of the instrument and of its industrial applications were published by Shakespear (7) and Daynes (2) respectively in the following year.

The katharometer has been made in several forms, but in the types

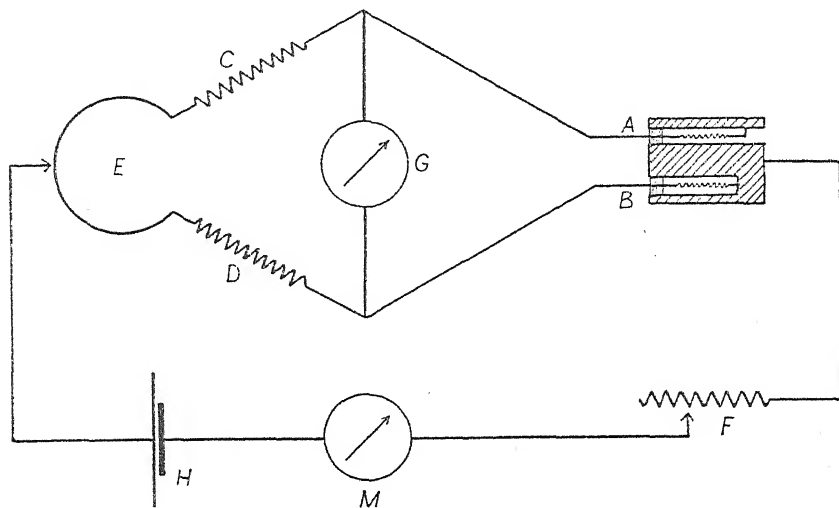


FIG. 1. General circuit diagram of katharometer. A, open spiral; B, control spiral; C and D, 10 ohm manganin resistances; E, manganin loop for balancing; F, heating current rheostat; G, galvanometer; H, accumulator; M, milliammeter.

most generally used the instrument consists of a cylindrical copper block about 4 cm. long and 2.5 cm. in diameter in which are drilled parallel to the axis two cylindrical holes each about 0.6 cm. in diameter extending from one face nearly to the other. Each of these holes contains a spiral of very thin platinum wire, having a resistance of about 10 ohms. The ends of these spirals are attached to pairs of copper leads. In some instruments both leads from each spiral are insulated from the block and brought to the outside, while in others only one lead from each spiral is insulated and brought out, the other lead being connected to the block so that the latter forms part of the electrical circuit. The spirals form two adjacent arms of a Wheatstone's bridge, and are exposed to the gas under examination and to the control gas respectively. The other two arms of the bridge are composed of manganin and also have each a resistance of about 10 ohms. The general circuit of this instrument is shown in Fig. 1.

In what we have called in a subsequent section of this paper the commercial type of instrument, one of the katharometer chambers (the open or experimental chamber) communicates directly with the gas outside the katharometer block by means of three small holes, the other communicates by means of a copper tube to any closed vessel containing any desired control gas mixture. The two manganin arms of the bridge are adjustable to some extent (see Fig. 1), so that the bridge can be brought into approxi-

mate balance. The manganin resistances are enclosed inside an iron case, which is attached to the katharometer block, so that the whole arrangement is very portable.

In another type of instrument the experimental chamber communicates with the gas outside by three small holes as in the commercial type, but the control chamber is hermetically sealed. Such an instrument, without the manganin balancing coils or the outer iron case, was used by Slater in his work on the respiration of the cockroach, and we have therefore, for the sake of brevity, spoken of this as the Slater type of instrument.

In another form of the instrument the katharometer block contains four platinum spirals each forming one arm of a Wheatstone's bridge. Two spirals forming opposite arms of the bridge are in contact with the gas of unknown composition, the others with the control atmosphere; the arrangement in this respect is similar to that of Koepsel. This is spoken of by the Cambridge Instrument Company who make it as the specially sensitive type of katharometer.

In yet another form the katharometer block contains four holes drilled parallel to the axis of the block, but only two contain platinum spirals. Each of the other holes communicates with one of the chambers containing the spirals, and through them the two gases to be compared are passed. This is known as the double-flow katharometer, and is said to be useful for comparing two gases of slightly different composition.

The current through the bridge in this, and in all other types of apparatus embodying the same principle, must be kept constant, as changes in the current strength must result in alterations in the temperature and resistance of the platinum spirals. The sensitiveness of the instrument is, indeed, very nearly proportional to the cube of the current. The recommended current strength is 100 to 120 milliamperes.

The measurement is usually made by observing the deflexion of the galvanometer, but the balanced bridge arrangement may be used in which the resistances of the balancing arms are adjustable and the change in resistance required to bring the galvanometer deflexion back to zero is measured. The apparatus was used in this way by Slater in his work on the cockroach already mentioned.

The arrangement of Weaver, Palmer, Frantz, Ledig, and Pickering. The arrangement of Weaver and his co-workers appears to have been developed at the U.S.A. Bureau of Standards during the years 1918 and 1919, while a description of the apparatus was published in 1920. The arrangement is essentially the same as that of the katharometer, but instead of spirals of thin platinum wire lengths of it are stretched along the axes of the gas chambers. These chambers are constructed so as to allow of the passage of a continuous gas stream through them. It is thus possible to pass the gas stream through the experimental chamber, then to remove

the constituent the concentration of which is to be determined, and finally pass the residual gas through the comparison chamber. The balancing arms of the bridge take the form of a slide wire with resistance boxes at each end. The measurement is made by the balanced bridge method.

The apparatus of Noyons. Noyons' apparatus, described in 1922, was designed especially for determining respiratory carbon dioxide. It resembles that of Weaver and his collaborators in that stretched fine platinum wires were used, and not spirals, but the galvanometer is read directly, and not the change in resistance required to bring the bridge back into balance.

In Noyons' apparatus a galvanometer deflexion of 1 mm. at 1 metre corresponded to 0.033 per cent. carbon dioxide. As will appear from the sequel, the sensitivity of his apparatus is therefore very much less than that of which the katharometer is capable. The apparatus is, however, noteworthy, as it appears to be the first in which the principle of the katharometer was utilized for biological work.

INSTRUMENTS USED.

In the course of the present work four types of katharometer were used, and their performance thoroughly investigated as regards their suitability for work on respiration. The first two were (*a*) the ordinary commercial type of instrument as used by Waller (10), and also apparently by Gregory and Richards (3), and (*b*) the Slater pattern (8), but differing apparently from the one used by Slater in that it had a third chamber bored in the katharometer block, and communicating with the chamber containing the control spiral. By enclosing in this chamber either a wad of moist cotton wool or a tube of phosphorus pentoxide, the air surrounding the control spiral could be kept in either a moisture-saturated or a dry state as required.

The commercial type of instrument, with its external control chamber and with its iron box containing the bridge and balancing resistances, need not be dwelt upon to any extent. It was found to be quite unsuitable for work of the degree of accuracy required in physiological investigations. This was because its design was such that it could not be totally immersed in water in a constant temperature bath, thus rendering too difficult the control of such disturbing factors as thermo-electric effects in the bridge wires and connexions and changes in humidity of the air surrounding the control spiral. This latter was made still more difficult by the presence of an external control chamber communicating with the control-spiral chamber in the katharometer block by means of a tube. Further, the connexion between the plant chamber and the open-spiral chamber of the katharometer by means of a tube was, owing to the diffusion lag produced (to give one obvious reason), quite unsatisfactory. It is clear that Gregory and Richards entirely over-

looked or failed to realize the more important of these points, the significance of which will appear later.

The Slater type of katharometer was the one used in the preliminary stages of this work commenced in Reading. It was purchased from the Cambridge Instrument Company with the aid of a grant from the Research Board of the University of Reading, and was kindly lent to us by that body for further work carried out at Birmingham. This instrument was much better in its performance than the commercial pattern, but in spite of this it possessed drawbacks which led to its being discarded as a suitable instrument for use in the study of respiration. The advantages possessed by it over the commercial type were the already mentioned water or phosphorus pentoxide chamber, situated *in* the katharometer block, and the brass disc to which a plant chamber could be cemented. This instrument was first fitted up in the way described by Slater, and with the air in the control chamber kept dry with phosphorus pentoxide, but it was found that no steady reading could be obtained when using a galvanometer of the order of sensitivity required. The creep of the galvanometer reading first in one direction and then in another was found to be largely due to a number of thermo-electric effects. Thermo-couple effects were present at all the junctions between wires and the terminals of the resistance boxes and switches and the junctions between the katharometer wires and the attached leads. In addition to these, more serious disturbances were caused by changes in resistance due to changes in temperature of the wires of the external circuit which were first of all of No. 22 S.W.G. copper. The heat from one's hand when held near to one of these wires was sufficient to start the galvanometer spot drifting away from its original position. All the No. 22 connecting wires were replaced by thick ones of No. 12 S.W.G. copper, but although some improvement resulted the disturbances were still present. Finally No. 16 manganin wire was used, with still further improvement, but still the thermo-couple effects at joints and a certain amount of thermo-electric effect in the manganin wires existed and caused trouble. It thus became evident that the balanced bridge arrangement, as used by Slater, introduced undesirable complications. It was therefore decided to try the deflection method as used by Waller and Gregory and Richards. The instrument was therefore connected up in a similar way to that used in the commercial instrument (see diagram, Fig. 1). In order to eliminate all thermo-electric effects, all the connecting wires and the 10 ohm bridge coils were made of manganin, and a manganin loop with a movable connexion was incorporated for balancing purposes. All these components were attached to a glass framework which fitted into an inverted bell-jar having a neck that could be attached to the top of the katharometer by means of a piece of wide-bore rubber tubing. This bell-jar was filled with liquid paraffin and fitted with a mechanical stirrer which

kept the oil in circulation. The whole was then immersed in a large constant temperature water-bath. This arrangement when first fitted up gave perfectly steady galvanometer deflexions, but after it had been working for a few days the creep of the galvanometer spot began to make itself evident again. On the suggestion of Dr. Shakespear, the phosphorus pentoxide in the control chamber was examined, and was found to have absorbed sufficient water to liquefy it. It appears that water is able to diffuse through the copper of which the katharometer block is made, and consequently after the phosphorus pentoxide has absorbed all it can, the air in the control chamber gradually becomes saturated with water vapour, to which the katharometer is very sensitive.

The effects of change in the humidity of the air surrounding the control spiral were eliminated by replacing the phosphorus pentoxide by a wad of wet cotton wool, so that the control spiral, like the open spiral, was always in a moisture-saturated atmosphere.

From this point we were no longer troubled with the annoying, continual creep of the galvanometer spot across the scale—that is, the movement referred to by Gregory and Richards for some unknown reason as ‘zero drift’. The major difficulties connected with the use of the instrument were thus disposed of. There still, however, remained an unsteadiness in the galvanometer deflexion which, although insignificant compared with that arising from the above causes, was sufficient to render the katharometer unsatisfactory for work of any considerable degree of accuracy. This was due to a number of causes which were investigated and finally mastered, but as they are of a more general nature and not inherent in any particular type of katharometer they will be dealt with under a later section of this paper.

A serious drawback to this Slater pattern instrument was the fact that the brass disc, to which the plant-chamber had been cemented, formed an integral part of the block. This meant that in order to cement on the plant-chamber in a satisfactory manner the whole katharometer had to be heated up to about 100°C ., a drastic treatment which tended to change the characteristics of the platinum spirals, and which had a softening effect on the ebonite insulation surrounding the leads to the spirals. Then, again, the katharometer, attached as it was to the jar of oil containing the external resistances, &c., together with a mechanical stirrer, was a far too unwieldy piece of apparatus for convenience of manipulation.

At this point Dr. G. A. Shakespear, who has given us much help and advice in the course of this work, provided us with a katharometer which he modified in various ways to meet our requirements. This instrument had screwed to its upper end a copper tube which contained the 10 ohm manganin resistances, and from which the manganin loop for balancing purposes, to which one battery lead was attached, projected (see Fig. 2).

A threaded brass disc, to which the plant-chamber could be satisfactorily cemented, screwed on to the lower end of the katharometer block. Greased leather washers were used to make water-tight joints between the brass disc

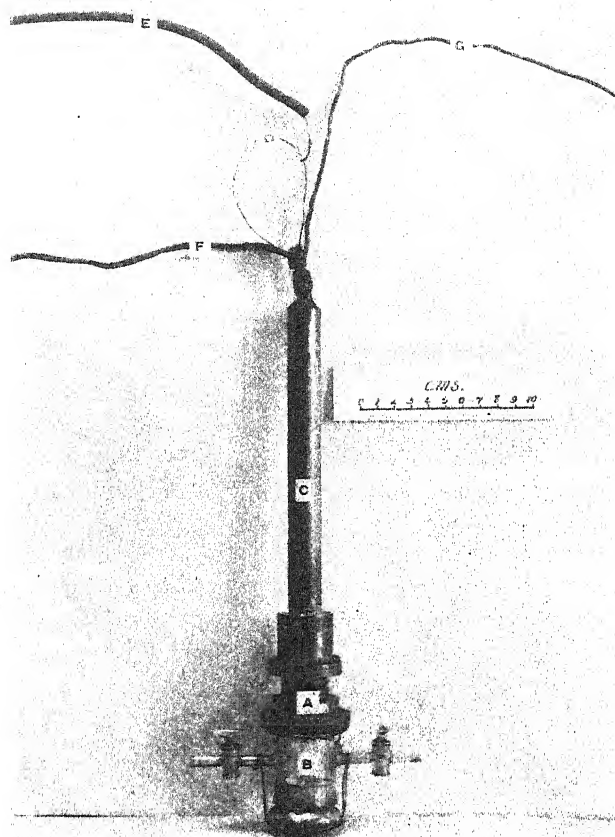


FIG. 2. Special katharometer constructed by Dr. G. A. Shakespear. A, katharometer block; B, plant-chamber; C, tube containing resistances; D, manganin loop for balancing; E and F, battery leads; G, twin galvanometer lead.

and the copper tube and collars screwed on to the katharometer block. This instrument, together with its plant-chamber, and the greater part of the tube containing the resistances, could be immersed in water in a constant temperature bath, and its compactness made it very easy to handle and to work with. It possessed, however, one serious defect which rendered it unsuitable for work where its immersion in water was necessary. This was the absence of a water-chamber in connexion with the control spiral chamber for the purpose of keeping the air in that chamber saturated with

moisture. The result of this was that after it had been immersed in the water-bath for some days the galvanometer creep began. This was caused by moisture diffusing through the block into the control-chamber as de-

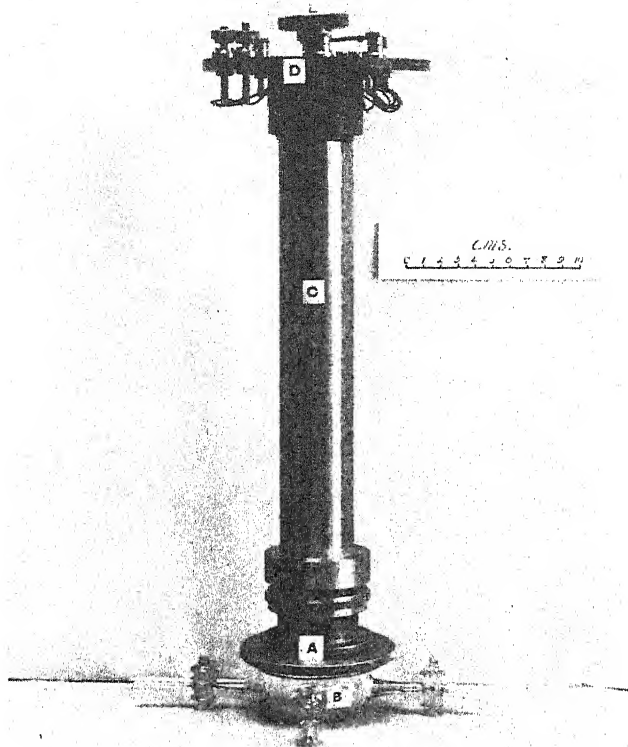


FIG. 3. Katharometer with balancing switch. A, B, and C as in Fig. 2; D, ebonite disc carrying switch and terminals; E, operating knob of switch.

scribed above. In spite of this, however, the Shakespear instrument was an important step forward and led directly to the design of our final pattern.

The katharometer used in our final pattern instrument is marked A in Fig. 3, and was made specially for us by the Cambridge Instrument Company. In this instrument the block is of a larger size than that used in the commercial instruments, its diameter being 4.5 cm., and there is a centrally placed water-chamber communicating with the control spiral chamber. A number of interchangeable brass discs to which the plant-chambers can be cemented screw on to the base of the block and make a gas-tight joint with it. Both leads, from each platinum spiral, are of No. 16 manganin wire and come out through the insulating plugs, so that

the block itself forms no part of the electrical circuit as is the case in the two instruments described above. In those the return ends of the spirals were connected to the block, to which in turn one battery lead was connected.

This katharometer block was then fitted up as follows:—

A piece of $1\frac{3}{4}$ -inch-bore brass tube, $10\frac{1}{2}$ inches long, was fitted at one end with a gland which fitted the katharometer block. This gland when packed with string and red lead and tightly screwed up made a secure water-tight joint between the tube and the block (Fig. 3). An ebonite ring was turned up to fit the upper end of the tube and was cemented to it. To this ring an ebonite disc was attached by means of three screws, and on the disc were mounted a switch-arm and eight contact studs. The brass tube, as in the Shakespear instrument, forms a housing for the external bridge circuit. The external bridge resistances were 10 ohms each, and were wound non-inductively, with No. 32 S.W.G. double silk covered manganin wire, on ebonite bobbins.

While working with the Shakespear instrument it was realized that if a galvanometer were used that was sufficiently sensitive to record the small changes in carbon dioxide concentration with which we were likely to be dealing, the useful range of the instrument would be very small. An increase in carbon dioxide concentration of approximately from 0 per cent. to 2 per cent. in the air in the plant-chamber caused the galvanometer spot to travel right across a 50 cm. scale. It was, therefore, necessary to incorporate in the instrument a device whereby the electrical balance could be altered by a number of steps, each step to cause a galvanometer deflexion across the scale in the reverse direction to that caused by increase in carbon dioxide in the plant-chamber. In order to accomplish this the two return leads from the spirals were soldered together so as to form a loop, and eight tappings were taken from this loop by soldering lengths of No. 22 double cotton covered copper wire to it. The intervals between these tappings were arranged so that by changing the battery connexion from one tapping to the next the galvanometer deflexion was caused to move back across the scale through a distance of about 30 cm., which roughly corresponded to a decrease in carbon dioxide percentage of 1.6 in the air surrounding the open spiral. The tappings were connected consecutively to the contact studs on the ebonite disc so that, with the battery lead connected to the switch arm, the galvanometer deflexion could be brought back near to zero eight times while the percentage of carbon dioxide in the plant-chamber rose to about 12, a value which we considered high enough for our present needs.

A circuit diagram of the instrument is given in Fig. 4, the battery and galvanometer leads being brought up to terminals situated on the ebonite disc as shown. A pair of additional terminals were added having connexions

similar to those of the galvanometer terminals. These were fitted for convenience of connecting up in case it was desired to experiment with the balanced bridge method of working at any time.

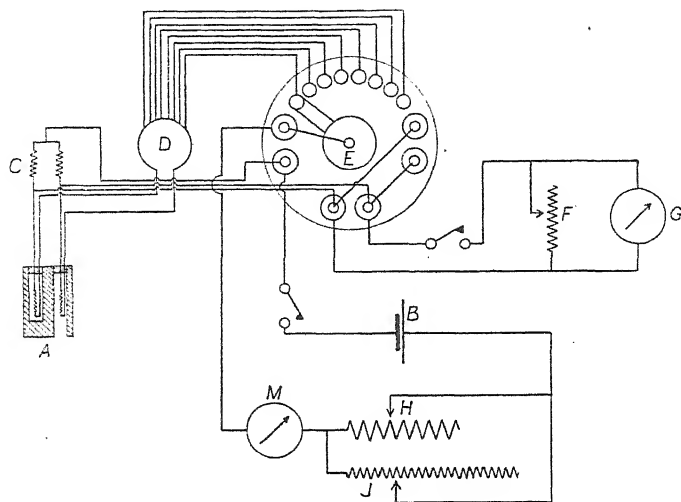


FIG. 4. Circuit diagram of katharometer with balancing switch. A, katharometer block; B, accumulator; C, 10 ohm manganin resistances; D, manganin loop; E, balancing switch; F, galvanometer shunt; G, galvanometer; H, 10 ohm rheostat; J, 1,250 ohm rheostat; M, milliammeter.

Sources of error and difficulty. In addition to the sources of error and difficulty mentioned above, viz. thermo-electric effects due to temperature variations of leads and junctions in the bridge circuit, and the effect of variation in the concentration of water vapour in the air surrounding the two katharometer spirals, a number of others were encountered and had to be eliminated. These will now be dealt with.

Variations in the temperature of the bath in which the katharometer was immersed were found to have a slight direct effect apart from any indirect effect through changes in water vapour concentration. This effect was determined in our final pattern katharometer by raising the temperature of the bath very slowly so that the air surrounding both spirals had time to take up water and maintain a saturated condition. By reading the galvanometer deflexion at intervals during this process it was found that a rise of one centigrade degree at temperatures between 17° and 25° C. caused a change of deflexion of 3.8 mm. in a direction opposite to that caused by increase of carbon dioxide concentration in the open-spiral chamber. This, with the sensitivity we were using at the time, has the same effect as a reduction of carbon dioxide concentration in the plant-chamber by about 0.025 per cent.

Another point that has to be kept in mind when working with the

katharometer is that the action is only quantitative. Consequently other gases besides carbon dioxide composing the atmosphere in the plant chamber have their effect. As this matter is one of considerable complexity, it is dealt with at some length in a later section.

The effect of changes in the pressure of the atmosphere inside the plant-chamber was found to be rather curious. A slight change of pressure, as observed by Waller, caused a relatively large but only momentary change in galvanometer deflexion. In addition to this transitory effect, however, change in pressure does, as stated by Gregory and Richards, have a small permanent effect. In the case of our instrument an increase of pressure of 0.01 atmospheres caused a permanent change in deflexion of 3 mm. in the same direction as that caused by increase in carbon dioxide concentration. It would appear that Waller's apparatus was insufficiently sensitive to show the permanent effect, while Gregory and Richards did not observe the transitory deflexion or were using a very heavily damped and consequently sluggish galvanometer.

A further question, and one which raises serious criticism against Slater's apparatus in which the katharometer is mechanically rocked to and fro in his water-bath, is the effect of shaking. Shaking the instrument is liable permanently to change its characteristics by causing a deformation of the delicate platinum spirals. This was forcibly demonstrated to us when working with the Slater instrument. During our experiment the katharometer was accidentally jarred slightly, with the result that the galvanometer deflexion immediately showed a change of 80 mm. It can easily be understood that an effect of this kind might upset a series of experiments by rendering recalibration necessary.

Another serious source of error that we experienced was the effect of slight fluctuation in the heating current supply to the katharometer. Even when several large capacity accumulators connected up in parallel were used, it was found that slight changes in current repeatedly occurred. These changes caused marked corresponding changes in galvanometer deflexion, which vary in magnitude with different katharometers. To give two examples of this: the Shakespear instrument, the working current of which was 100 milliamperes, showed a change of galvanometer deflexion of 50 mm. for a change of 1 milliampere when the current was varied between 90 and 110 milliamperes. Our instrument, which worked at 120 milliamperes, gave a change in deflexion of 23 mm. per milliampere when the current was varied between 115 and 122 milliamperes.

Finally, there is the diffusion lag between the open spiral and the air in the plant-chamber. The effect of this is that a given time must elapse between a change in the concentration of the carbon dioxide in the plant-chamber and the completion of the change in galvanometer deflexion produced by that change. Naturally this will depend chiefly upon the

size of the plant-chamber and the nature of the diffusion path leading to it from the open katharometer spiral. In our instrument a single hole 1.5 mm. in diameter, covered by a piece of gauze, led into the open spiral chamber, and the lag period was 4.25 minutes. In the Shakespear instrument there were three small holes each 0.75 mm. in diameter and the lag period was 4.5 minutes. Although one would naturally expect that the arrangement used by Gregory and Richards with its long diffusion path would show a longer lag period than those possessed by the instruments we used, the lag period which they mention as being considerably over an hour must have been largely due to causes independent of the instrument.

GENERAL DESCRIPTION OF THE APPARATUS.

A number of points of importance with regard to the use of the katharometer for respiration experiments and the subsidiary apparatus necessary for such work will now be considered in turn.

Electrical circuits. The first circuit tried, as already mentioned, was the balanced bridge arrangement as used by Slater. This circuit, for reasons already stated, was found to be unsatisfactory. The method of working is briefly described on p. 463, and by its means the actual change in resistance of the open spiral, due to change in carbon dioxide concentration in the air surrounding it, is measured. With such an arrangement calibration of the instrument aims at correlating definite resistance values of the open katharometer spiral with known concentrations of carbon dioxide in the plant-chamber.

The arrangement, which we found satisfactory, and which we finally adopted, is in some measure a combination of the above-described balanced bridge method and the unbalanced bridge method, in which changes in the gas surrounding the open spiral are measured simply by noting the galvanometer deflexion produced. The latter method, of course, must be used if one works with the commercial pattern katharometer. It will be clear if Fig. 4 is examined that the device we have incorporated in our instrument for the purpose of increasing its range is simply an arrangement whereby the bridge may be roughly balanced a number of times as the galvanometer deflexion continues to increase with a continuous increase of carbon dioxide in the plant-chamber. We thus have the simplicity of reading which is a feature of the commercial instrument, coupled with the wider range in carbon dioxide concentration with which the balanced bridge method is theoretically capable of dealing.

Galvanometer. We have found the selection of a suitable galvanometer essential for the successful operation of the method. Previously published accounts of the katharometer itself, and of biological work attempted with its aid, are quite unhelpful in regard to this point. For use with their balanced bridge arrangement Weaver and his collaborators

at the U.S. Bureau of Standards merely state that they used a portable inexpensive d'Arsonval galvanometer to indicate the point of balance. Noyons' description of his Kipp galvanometer is confined to the statement that it is very rapid and allows a reading to be made in one-tenth of a second. Neither Slater nor Gregory and Richards describe their galvanometers, although the former, who used a balanced bridge arrangement, mentions that the galvanometer gave a deflexion of 1 mm. at one metre when the balance in one of the manganin arms was thrown out by 5×10^{-6} ohms. Waller alone records that he used a moving coil galvanometer of 21 ohms resistance giving a deflexion of 125 mm. per microampere at 50 cm.

After a trial of several galvanometers we found a Broca (moving magnet) galvanometer manufactured by the Cambridge Instrument Company very suitable for use in the katharometer circuit. The advantages of this type of instrument are relatively quick movement and the ease with which the sensitivity can be altered and the damping regulated. The instrument used by us had two coils of resistance 4.90 and 4.95 ohms respectively. In our work we have used these connected in series. The sensitivity was adjusted so that a current of 1 microampere produced a deflexion of 61 mm. at 1 metre. The period of the instrument was 1.2 seconds. The sensitivity of our galvanometer, as we used it, was thus much less than Waller's. Even so, its sensitivity was much greater than we required, and we accordingly reduced it by shunting with a resistance which could be varied from 1 to 10 ohms. In this way a range of sensitivities could be obtained at will with the greatest ease. Actually, working with a 5 ohm shunt, a galvanometer deflexion of 1 mm. corresponded to a change in carbon dioxide concentration of 0.007 per cent., practically the same as that recorded by Waller (1 mm. equivalent to 0.006 per cent. carbon dioxide) with his very much more sensitive galvanometer. The divergence between the sensitivity of our system and that of Waller's is inexplicable unless Waller's katharometer was much less sensitive than ours, or unless he had an unmentioned resistance in series or in parallel with the galvanometer.

The advantage of a galvanometric arrangement which permits a wide range of sensitivity is at once apparent, as it allows of the measurement of a correspondingly varying range of carbon dioxide concentrations. With our galvanometric arrangement kept constant as described above, and with a 5-ohm shunt, we were able to measure carbon dioxide concentrations of from 0 to 12 per cent. with an accuracy of 0.007 per cent., since a change of deflexion of 1 mm. under these conditions corresponds to a change in carbon dioxide concentration by this amount. By decreasing the shunt resistance to 1 ohm the sensitivity is correspondingly reduced, while by elimination of the shunt the sensitivity can be increased 2.19 times. Thus

by merely altering the galvanometer shunt a range of sensitivities can be obtained between 1 mm. deflexion, corresponding to 0.019 per cent., and to 0.0032 per cent. By altering the sensitivity of the galvanometer the apparatus can be made much more sensitive, but the accuracy of the measurements is limited by the accuracy with which the heating current can be kept constant, and hence by the sensitiveness of the milliammeter used for measuring this current. With the very good instrument used by us the limit of accuracy in carbon dioxide measurement imposed by the milliammeter is in the neighbourhood of 0.001 per cent.

It may be possible to use a moving-coil galvanometer in place of the Broca instrument, but one galvanometer of this type that we tried was found to be very heavily damped, so that the movement of the galvanometer was so excessively sluggish as to render its use impossible. But it should be possible to find a moving-coil galvanometer with a resistance of 10 ohms and with a sensitivity of 60 to 100 mm. per microampere with a rapid enough movement for work with the katharometer. We can at present, however, make no recommendations in this matter one way or the other.¹ The galvanometer can be made self-recording in the manner described by Waller.²

Milliammeter. Stress has already been laid on the necessity of maintaining a high degree of constancy in the current supply to the katharometer spirals. It is curious to note that in spite of the importance of this none of the authors we have cited as having attempted to use the katharometer for physiological purposes seem to have considered this point worthy of mention. We found that an ordinary small laboratory milliammeter of good quality, having a range of 0 to 120 milliamperes, when connected in the heating current circuit, was insufficiently sensitive and quite useless. With such an instrument we found that fluctuations of sufficient magnitude to cause changes in galvanometer deflexion of several millimetres could occur, and at the same time be too small to give any visible deflexion in the milliammeter. After trying a number of milliammeters we finally used a Cambridge 'Unipivot' pattern 'T' galvanometer, having a 170 mm. scale with a range of 0 to 150 milliamperes. On this we mounted a microscope which magnified 50 diameters and which had a cross wire in the eyepiece. The microscope was mounted so that the cross wire coincided with the centre of the image of the 120 division on the milliammeter scale when the latter was brought into focus. The pointer of the instrument was then focussed and the current adjusted until the cross-wire was exactly over its centre. This was done every time a galvanometer reading was taken.

¹ Since writing this, Messrs. Tinsley & Co. have made us a moving coil galvanometer with the resistance and sensitivity indicated. This instrument is very suitable for use with the katharometer.

² For some purposes we have adopted a different recording system which we propose to describe on a later occasion.

The adjustment of the current was made by means of a 10-ohm sliding resistance in the circuit and which was shunted by another sliding resistance having a maximum value of 1,250 ohms (H and J in Fig. 4). The current was roughly adjusted by means of the former and then accurately brought to its correct value by means of the latter with the aid of the microscope on the milliammeter. Slater's arrangement of regulating resistances was similar to that described above, but his shunt resistance had a maximum value of only 60 ohms.

Plant-chamber. The type of plant-chamber we adopted as being most generally useful for experiments upon relatively small amounts of tissue is shown attached to the katharometer in Fig 3. It is made of glass, which is obviously the only really satisfactory material for the purpose, and has a ground flange to enable it to be securely cemented to the katharometer disc. Its internal dimensions are 4.5 cm. in diameter and 2.5 cm. deep, and it has three glass tubes with taps fused into it as shown. The middle one of these tubes is a capillary for the attachment of a manometer, while the other two, situated at opposite sides of the chamber, have bores of 2.5 mm. and are for the purpose of conducting gas to or from the chamber as desired.

Temperature control of the instrument. The katharometer with its attached plant-chamber, when under working conditions, was immersed to within 5 cm. of the ebonite disc carrying the switch, in a 40-gallon water-bath. This was electrically heated and kept at 25° C. The temperature was kept constant to within 0.05° C. by means of a mercury regulator and two relays. The details of this constant temperature apparatus have already been published by one of us (Leach, 1931).

CALIBRATION.

The question of calibration of the katharometer requires careful consideration. Since the change in concentration of any one constituent of a mixture of gases may result in a change in the thermal conductivity of the mixture, it is necessary to consider separately the calibration of the instrument for the different gas mixtures that may be involved in work on respiration. One complication, however, is met with whatever may be the gaseous system involved, namely, the effect of water-vapour. Since differences in concentration of water-vapour affect the thermal conductivity of a gas containing this substance, it is necessary to ensure that no change in concentration in water-vapour occurs in either of the katharometer chambers. This can only be ensured by keeping the chambers either completely dry or saturated and at constant temperature. It has already been shown that it is impracticable to use dry air in the katharometer, and consequently it is essential to calibrate and use the instrument with both chambers saturated with water-vapour. Neither Slater nor Gregory and

Richards make any mention of the possible disturbing effect of water-vapour, but Waller states that in his arrangement both chambers are kept saturated with moisture by the presence of a little water. But he also makes the surprising statement that he was unable to detect any difference between the action of air which has been bubbled through water and air which had passed through dehydrating agents. This absence of effect of change in water-vapour content is completely at variance with our own experience. We find that unless care is taken to ensure a saturated atmosphere in both chambers water-vapour may be a most troublesome factor.

Another factor, although one much less disturbing than water-vapour, which may arise, and should be eliminated, is change in pressure. Should the respiratory quotient be greater or less than unity, the number of molecules of carbon dioxide evolved will not be the same as the number of oxygen molecules absorbed, and in a closed plant-chamber a change in pressure will result, which, as already shown, has some effect on the galvanometer deflexion. It is therefore necessary to ensure that measurements are taken at constant pressure, and some device, such as the manometric arrangement used for this purpose by Slater, may be necessary. Waller, as already noted, concluded that change in pressure has no permanent effect, a conclusion with which we do not agree. Gregory and Richards, on the other hand, were aware of the effect of change in pressure but concluded that this effect was too small to affect their results appreciably, and apparently they made no attempt to eliminate it. We do not find that the effect of pressure change is necessarily negligible, and in calibrating and using the instrument the galvanometer readings should always be taken with the gas in the experimental chamber at constant pressure.

We will now proceed to consider the various gas systems likely to be met with in respiration work and the special questions raised by the calibration of the instrument with these different gas systems.

Oxygen-carbon dioxide mixtures. The simplest system to be met with in respiration work is one in which the respiring tissue is in contact with a gas mixture containing only the two gases oxygen and carbon dioxide. This was the case in Slater's work with the cockroach (9). Here all that is necessary in calibrating the instrument is to determine the galvanometer deflexion given by mixtures of oxygen and carbon dioxide of known composition. These may be obtained by mixing together known volumes of the two gases, or by the respiration of some suitable material in oxygen and determining the proportion of carbon dioxide in the resulting gas mixture by means of a Haldane gas analysis apparatus. Analysis of a number of such gas mixtures with observation of the galvanometer deflexions corresponding to them should be made to ensure adequate calibration over the range of carbon dioxide concentrations likely to be met with.

Nitrogen- (or Hydrogen-) carbon dioxide mixtures. Determinations of respiration are frequently required under conditions of complete absence of oxygen. In such cases it is usual to allow the tissue to respire in an atmosphere of pure nitrogen or pure hydrogen. Under such conditions carbon dioxide is evolved, but none of the external gas is absorbed by the tissue, consequently there will be an increase in the mass of the gas in the plant-chamber, and the pressure will require adjustment. As only two gases are present calibration can be effected in just the same way as in the previous case.

Aerobic respiration in air with a respiratory quotient of unity. A very usual system is that of tissue respiring aerobically in ordinary air. The system is more complex than those already considered in that three gases—nitrogen,¹ oxygen, and carbon dioxide—are involved. But if the respiratory quotient is unity, the oxygen removed is exactly replaced by carbon dioxide, and the proportion of the nitrogen remains unchanged. Actually, therefore, calibration is effected in the same way as in the case of simple oxygen-carbon dioxide mixtures, for the third constituent, nitrogen, remaining constant, has no effect on the galvanometer deflexion.

Respiration in air with a respiratory quotient different from unity. In cases where respiration takes place in air, and the respiratory quotient is not unity, the changes in gas composition during respiration are more complex. For since the number of molecules of oxygen absorbed differs from that of the carbon dioxide evolved, there will be a change not only in the proportion of these gases, but also of the nitrogen in the plant-chamber. A numerical example will make the situation clear. Let us, for the sake of simplicity, suppose that we begin with an atmosphere containing 20 per cent. oxygen and 80 per cent. nitrogen, and that the tissue respiring in this atmosphere has a respiratory quotient of 0.5—that is, for every molecule of carbon dioxide evolved two molecules of oxygen are absorbed. When one-tenth of the oxygen originally present has been absorbed the gas is now made up of oxygen, nitrogen, and carbon dioxide in the proportions 18:80:1, and the respective percentages of these gases are therefore 18.18:80.81:1.01. The result is that after adjustment of pressure the concentration of every gas in the system has altered; oxygen has decreased by 1.82 per cent. of the total volume, nitrogen has increased by 0.81, and carbon dioxide has increased from zero to 1.01. The changes in the system thus amount to a replacement of oxygen (1.82 parts) partly by nitrogen (0.81 parts) and partly by carbon dioxide (1.01 parts). The galvanometer deflexion is thus due to the sum of changes in thermal conductivity brought about by the replacement of oxygen by carbon dioxide and by the replacement of oxygen by nitrogen. Hence, if the instrument has been calibrated for carbon dioxide concentrations by means of oxygen-carbon dioxide mixtures, or by mixtures obtained by respiration of tissue

¹ Argon, and other inert gases, may be regarded as forming part of the nitrogen (cf. p. 481).

in air, where the respiratory quotient is unity, the same calibration can only be employed in the case under consideration if the replacement of oxygen by nitrogen has no effect on the thermal conductivity. If this is not the case it will be necessary to apply a correction for the effect of the replacement of oxygen by nitrogen.

As a matter of fact the most recent determinations show that oxygen and nitrogen have almost exactly the same thermal conductivity. Thus Günther in 1906 found for both oxygen and nitrogen at 0° C. the same value, namely, 0.05694 (measured in calories $\times 10^{-3}$ per cm. per degree per sec.). Eucken in 1911 and 1912 found a value 0.0572 for oxygen and values of 0.0568 and 0.0566 for nitrogen, the values for the two gases differing by something in the neighbourhood of 0.9 per cent. Somewhat more divergent values were found by Weber in 1917, his values for a temperature of 0° C. being 0.05768 for oxygen and 0.0566 for nitrogen, a difference of about 2 per cent.

If we accept these values for thermal conductivity, and to this we have no alternative, we must conclude that a replacement of oxygen by nitrogen in the katharometer has no effect, or a comparatively small one. Consequently in a gas system containing oxygen, nitrogen, and carbon dioxide, with tissues respiring with a respiratory quotient less than unity, the only change in gas concentration likely to affect the katharometer appreciably is the change in carbon dioxide concentration, and the calibration of the instrument with oxygen-carbon dioxide mixtures is applicable to this more complicated case. Similar considerations hold if the respiring tissue has a respiratory quotient greater than unity. It is, of course, necessary to take account of the change in volume resulting from pressure adjustment when calculating the quantity of carbon dioxide evolved.

It has been shown that recent determinations indicate that the thermal conductivity of oxygen and nitrogen are very nearly the same, the divergence varying according to the observer between 0 and 2 per cent. In the latter case a substitution of nitrogen by oxygen would have a little less than one-twentieth of the effect of substitution of nitrogen by carbon dioxide. It is easy to calculate the error introduced in determinations of respiration in air when the respiratory quotient differs from unity if a calibration by means of oxygen-carbon dioxide mixtures has been employed. If the effect of substituting carbon dioxide in nitrogen is n times the effect of substituting oxygen in nitrogen, and if the respiratory quotient is C , the percentage error in the determination is—

$$80/n (1/C - 1).$$

It will be observed that the error is positive—that is, the result is too high, when C is less than unity, and negative when C is greater than unity.

Since it appears that n is not less than 20, the error introduced when the respiratory quotient is as high as 2.0 is not more than 2 per cent,

while with a respiratory quotient as low as 0.5 the results obtained are not more than 4 per cent. too high. With material respiring in air the divergence of the quotient from unity will not usually be as great as the examples here quoted.

In this discussion no account has been taken of the argon and other inert gases of the atmosphere. Since the argon will change in the same proportion as the nitrogen, these two gases may be regarded as one gas for our present purpose. The thermal conductivity of the nitrogen-argon mixture will actually be only a fraction of one per cent. less than that of nitrogen; the effect of the argon and of other inert gases present in still less quantity may therefore be neglected.

Both Waller and Gregory and Richards show some confusion of statement when dealing with the question of calibration. Waller makes Daynes responsible for the statement that 'the effect of O_2 upon thermal conductivity is only about one-tenth that of CO_2 '. Gregory and Richards also state that according to Daynes 'the effect of O_2 upon thermal conductivity is one-tenth that of CO_2 and in the opposite direction'. As it stands, this statement is difficult of interpretation, and we have been unable to trace it in either of the papers by Daynes that Waller cites, while Gregory and Richards do not mention Daynes in their literature list. From the context it may be supposed that what apparently Waller meant is, that the substitution of oxygen for air produces a change in conductivity which is one-tenth of that produced by the substitution of the same volume of carbon dioxide for air.

If we are correct in this interpretation of the statement attributed to Daynes it means that the difference in thermal conductivity between oxygen and air is about one-tenth of the difference in conductivity between air and carbon dioxide. Now recent determinations show differences in the thermal conductivities of oxygen and air little removed from zero. The determinations of the thermal conductivity of atmospheric air by Schwarze and Weber are respectively 0.0569 and 0.0568; recent determinations of the conductivity of oxygen are, as we have seen, 0.05694 (Günther), 0.0570 (Weber), and 0.05768 (Weber). The difference between the thermal conductivity of oxygen and air, as far as physical determinations allow us to judge, might be taken as in the neighbourhood of 0.8 per cent. that of air. The difference between the conductivities of air and carbon dioxide is about 40 per cent. that of air (air 0.05685, carbon dioxide 0.0336), so that, unless these more recent determinations of physicists are wrong, the effect of substituting oxygen for air cannot be anything like that assumed by Waller, and by Gregory and Richards after him.

It is just possible that Waller may have meant substitution of oxygen for nitrogen and not air. In this case similar considerations hold, since the thermal conductivities of air and nitrogen are nearly the same. But in this case

the correction of 10 per cent. proposed by Waller and applied by Gregory and Richards to their results obtained with aerobic respiration in air would be wrong; the results would be too high by 7.84 per cent., not by 10 per cent.

Determination of oxygen absorbed. If the apparatus is provided with a manometer, by which the pressure is adjusted and the change in volume observed, it is possible to calculate the amount of oxygen absorbed as well as the amount of carbon dioxide evolved. Such an arrangement was utilized by Slater in his apparatus and requires no redescription here.

The disturbing effect of other gases. If any gas apart from carbon dioxide should be evolved in respiration, measurements based on a calibration with oxygen- (air-, nitrogen-, or hydrogen-) carbon dioxide mixtures must necessarily be incorrect. Thus the evolution of alcohol vapour, which might take place in anaerobic respiration, would vitiate such measurements. Cases of this kind present special problems and call for special adaptation of experimental arrangements.

In this connexion it is to be noted that Gregory and Richards suspected the walls of their aluminium plant-chamber of giving off hydrogen, and, to prevent this, coated the inside of the chamber with shellac and white enamel. Such a treatment obviously requires careful control, as one would imagine that as shellac dried the solvent in which it was held, presumably alcohol, would evaporate into the chamber, and that the volatile medium in which the enamel was held would behave in the same way. It might be long before the last appreciable traces of these vapours ceased to be given off from the walls.

Experimental arrangement. The apparatus designed for a preliminary trial calibration of the katharometer is shown in its actual form in Fig. 5, and semi-diagrammatically in Fig. 6. For the purpose of our trial calibration it was decided to use germinating peas, as owing to their intense respiratory activity they furnish a ready means of quickly increasing the carbon dioxide concentration in the system. As the peas were used while in the early stages of germination, before rupture of the testa had occurred, the respiratory quotient would probably differ from unity. Reasons have already been given for concluding that within limits variation in the respiratory quotient does not affect appreciably the determination of carbon dioxide, but if it should be shown that oxygen and nitrogen differ more widely in their thermal conductivities than has been found by recent determination, it is clear that a general calibration by this method would have to be made with material having a respiratory quotient of unity.

It will be seen, if Fig. 6 is examined, that the plant-chamber B attached to the katharometer forms part of a circulatory system in series with the gas-circulating pump K, the respiratory chamber D containing the

peas, and the gas sampling tube C. The air is circulated round this system in the direction indicated by the arrows by means of the mercury pump K,

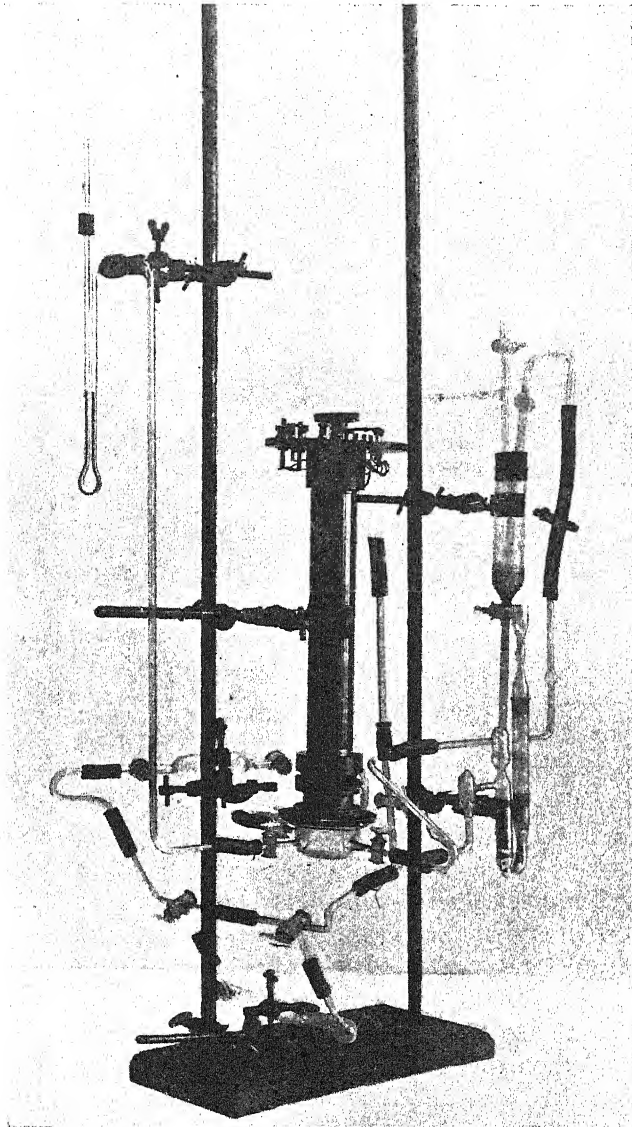


FIG. 5. Calibration apparatus. For description see text.

a description of which has already been published (Leach (4)). In carrying out the calibration, the following procedure was adopted.

After seeing that all rubber joints were secure, with the glass tubes pushed well into them to ensure a minimum of rubber being exposed to the

gas, a small quantity of water was introduced into the respiration chamber D. The two-way taps G and H were turned so that the respiration chamber was in the gas circuit, and the tap J in the tube F, which forms a communica-

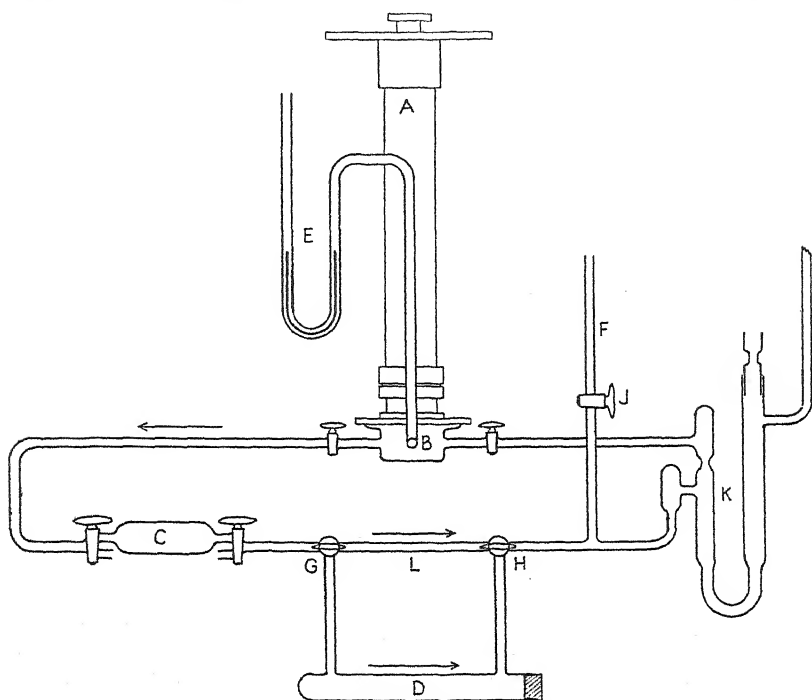


FIG. 6. General arrangement of calibration apparatus. For further description see text.

tion between the system and the outside air, was opened. The galvanometer and battery wires were then connected to the katharometer terminals and the current switched on and approximately adjusted to 120 milliamperes. This being done, the whole apparatus was lowered into the constant temperature bath and left for a few minutes to warm up. Tap J was then closed and the pump started, so that the air contained in the system was circulated round and round. After about ten minutes the pump was stopped, the katharometer current carefully adjusted by means of the microscope on the milliammeter and the variable resistances, and the galvanometer deflexion noted. This procedure was repeated at intervals of about ten minutes until the galvanometer showed a constant deflexion, which indicated that the water-vapour in the system had reached a state of equilibrium. The apparatus was then lifted out of the bath, and after closing the taps of the gas sampling tube this was removed from the circuit and replaced by another with its taps open. The respiration chamber was then filled with soaked peas and the apparatus again lowered into the bath; the air pressure in the system was adjusted to atmospheric pressure by

means of the tap J, and the manometer E which connected the plant-chamber, and the pump again started.

The air from the gas sampling tube, which had been removed from the circuit, was introduced into a Haldane gas analysis apparatus, and the percentage of carbon dioxide it contained was determined. Thus the first galvanometer deflexion, together with the corresponding carbon dioxide concentration in the air in the system, was obtained.

Meanwhile the respiring peas in the apparatus had increased the carbon dioxide content of the circulating air and caused a change in the galvanometer deflexion. The taps G and H were then turned so as to cut the respiration chamber out of the circuit, at the same time diverting the gas stream along the tube L, and tap J was opened and immediately closed to reduce the pressure inside the system to that of the atmosphere. The pump was again stopped, a galvanometer reading taken, and the gas sampling tube replaced by another containing fresh air as before. The respiration chamber was again connected into the circuit by means of taps G and H and the carbon dioxide content again allowed to increase. Meanwhile this second gas sample was analysed and the percentage of carbon dioxide present determined.

The above procedure was repeated until galvanometer deflexions corresponding to a number of carbon dioxide concentrations covering the full range of the katharometer were obtained. In observing the galvanometer deflexion with our apparatus, account must, of course, be taken of the actual stud used in the bridge balancing arrangement described on p. 471. The relation between the carbon dioxide concentration and galvanometer deflexion was found by Waller to be linear between the limits of 0.15 and 0.6 per cent. We have confirmed this, and, moreover, have found that the linear relation holds, with a very fair degree of approximation, up to at least 9 per cent. carbon dioxide.

Calibration in the manner described was carried out four times—that is, with four different samples of respiring material. The results of all four experiments are shown graphically in Fig. 7. Values obtained in the first, second, third, and fourth calibrations are indicated respectively by circles, crosses, triangles, and squares. The consistency is all that could be desired and affords a striking testimony to the reliability of the apparatus and method.

Since the relation between carbon dioxide concentration and galvanometer deflexion is linear the relation can be at once expressed in an equation. In the example given it is:

$$C = 0.0488 D - 0.140$$

where C is the concentration of carbon dioxide in per cent. and D is the observed deflexion of the galvanometer. The concentration of carbon dioxide may then be obtained either from the graph or the formula.

We would emphasize that this method of calibration was devised simply for preliminary trials, and was not intended to provide a permanent and final calibration of the apparatus. It has, however, the advantage that

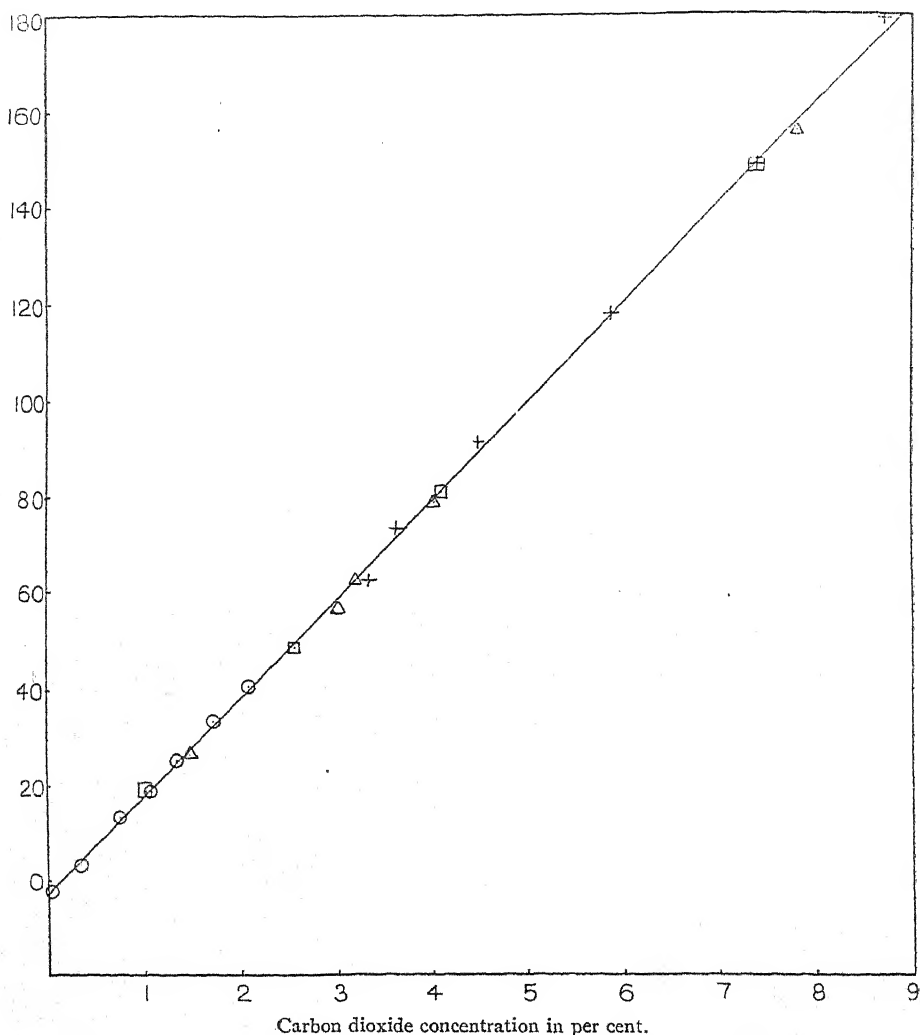


FIG. 7. Graph showing relationship between galvanometer deflexion and concentration of carbon dioxide in plant-chamber.

it provides atmospheres containing different concentrations of carbon dioxide readily and rapidly and may prove a sufficiently exact method for many purposes. But from what has been said earlier in this section it is clear that it may sometimes be necessary to calibrate in some other way, as, for instance, with gas mixtures obtained by directly bringing together pure gases in known proportions.

CONCLUDING REMARKS.

It has been our intention in this paper to show that the katharometer provides a method of measuring respiration which, in regard to exactness and sensitiveness, compares more than favourably with other methods, and which possesses the further advantages that both carbon dioxide and oxygen changes can be measured, and that a continuous record of carbon dioxide concentration in the plant-chamber can be obtained. The apparatus can, in fact, be made self-registering.

We have further endeavoured to indicate the difficulties and various sources of error which are likely to be met with in using the katharometer for measurements of respiration, and the ways in which these difficulties may be overcome and sources of error eliminated. To overcome these difficulties and eliminate the sources of error may require patience and care, but if the precautions we have indicated are observed the apparatus may be made as stable in behaviour as any other piece of physical apparatus. An arrangement in which the galvanometer deflexion drifts for no apparent reason should never be tolerated, for apart from the irritation produced by working with such a system, it is an indication that there are unknown sources of disturbance in the apparatus which should be tracked down and eliminated.

The apparatus, as we have described it, is adapted for use with a closed respiration chamber. Whether in any particular investigation it is desirable to use a closed chamber—a chamber forming part of a closed circulating system, or a chamber through which flows a continuous stream of gas from an external source—will depend upon the character of the problem under investigation. There appears to be no reason why the katharometer should not be usable for work on respiration where a circulatory system or a continuous gas stream is employed; indeed, the apparatus evolved by Weaver and his colleagues at the U.S. Bureau of Standards was designed for use in the latter way.

In conclusion we would record our thanks to the Research Board of the University of Reading, firstly, for a grant out of which one of the katharometers used in this work was purchased, and, secondly, for the loan of this instrument for our use in the University of Birmingham; to Dr. W. K. Slater for useful information in the early stages of this work; and finally to our colleague Dr. G. A. Shakespear, the inventor of the katharometer, but for whose generous advice in the later stages of the work the successful development of the apparatus described in this paper might have been long delayed.

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Observations on the Fat Metabolism of Leaves.

I. Detached and Starved Mature Leaves of Brussels Sprout (*Brassica oleracea*).

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I. INTRODUCTION.

IT is well known that the reserve fats in seeds undergo metabolism during germination, giving rise, among other products, to carbohydrates. It is also probable, from the fall in the respiratory quotient to well under unity in such cases, that the reserve fat is contributing carbon dioxide for respiration. In the case of the lower organisms, Stephenson and Whetham (10) have shown that Timothy grass bacillus can synthesize fat readily from sugar, and that when the supply of this is exhausted the fat rapidly disappears. The change in the respiratory quotient at the same time suggests that the fats were being utilized for respiration.

With regard to the role of the fats in the metabolism of the green leaf the available evidence is very meagre. This can be ascribed to two causes. In the first place the extraction of fatty material has hitherto necessitated the preliminary drying of the leaves, and has given extracts so heavily contaminated with leaf pigment that they could not be considered a measure of 'fat' or 'lipoid', in the somewhat loose sense that these terms have been used by earlier workers to denote the content of the ether or light petroleum extract of plant material. In the second place earlier workers considered that the amount of fat in a leaf is much less than that of the consumable sugars; consequently under conditions of starvation the latter would be used first in respiration. Published data for the respiratory quotient of detached leaves certainly support this latter contention.

Within the last few years methods of extracting the ether-soluble material from fresh leaves have been worked out by Chibnall and Channon (3), who have applied them to cabbage and have investigated in considerable detail the true fats and other ether-soluble materials present. (See Table I and later.) Further work (unpublished) carried on in this laboratory has shown that in certain samples of cocksfoot (*Dactylis glomerata*) the ether extract can be as high as 7 per cent., the true fat 2.9 per cent., and waxes 1.4 per cent. of the dry weight of the leaf. In these same leaves the total reducing sugar was only 3 per cent. of the dry weight. The surmise, then, that the fat content of a leaf is always much less than that of the sugar certainly does not hold in all cases. It is with the object of adding to our knowledge of the general fat metabolism of the green leaf that the experiments to be described in this paper were planned. Further experiments with widely different types of leaves, starved under different sets of conditions, will be necessary before any very general conclusions can be drawn. This paper, therefore, is to be looked upon as the first of a series dealing with the general fat metabolism of leaves, and it has been thought advisable to preface it with an account of the newer knowledge of the leaf fats and phosphatides and the change that these might be expected to undergo during the starvation of the leaf if their metabolism is analogous to that observed in the animal organism.

TABLE I.

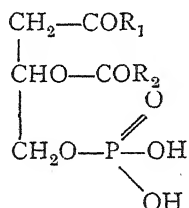
Ether-soluble Substances of Cabbage Leaves.

(Figures given are in percentages of total ether-extract.)

	%.
<i>Pigments.</i>	
Chlorophyll (a and b)	9.3
Carotene	0.5
Xanthophyll	0.8
<i>Substances containing phosphorus.</i>	
Calcium phosphatide	18.4
Unidentified calcium salts, possibly of fatty acids and phosphoric acid	5.0
Unidentified iron compound	3.0
<i>Glycerides and waxes.</i>	
Containing palmitic, stearic, linolic, and linolenic acids	17.5
Glycerol	1.3
<i>Unsaponifiable material.</i>	
Saturated fraction (crude hydrocarbon) chiefly nonocosane and di-n-tetradecyl ketone	12.3
Unsaturated fraction	
Sterols (by digitonin)	4.5
Unidentified liquid products, probably alcohols and hydrocarbons	13.3
	85.9

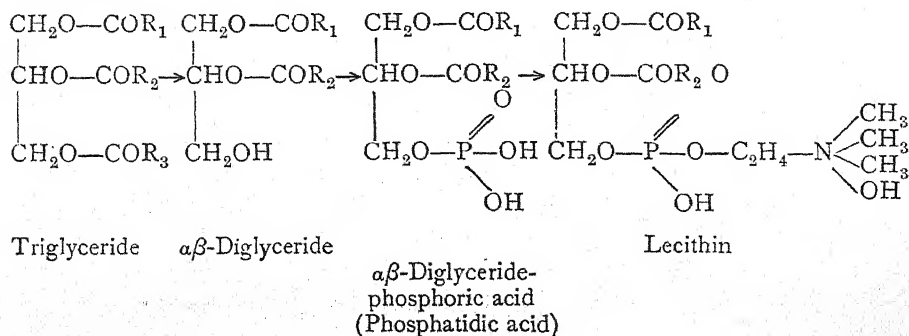
Table I shows the composition of the ether-extract of the cytoplasm of cabbage (*Brassica oleracea* var.) as found by Chibnall and Channon (3). This does not represent the whole ether-extract of the leaf, because the material referred to in the present paper as the cell-residue was not, in that research, extracted with ether at all.

It will be observed that the glycerides and phosphatides make up only about 40 per cent. of the ether-extract; the remainder is made up of pigments and unsaponifiable material, the major part of which on analysis was found to consist of the paraffin hydrocarbon nonacosane $C_{29}H_{60}$ and the closely allied di-n-tetradecyl ketone $CH_3(CH_2)_{13}CO(CH_2)_{13}CH_3$. From the point of view of the fat metabolism in the leaf the most interesting result was the discovery of the chemical nature of the phosphatide present. Lecithin, cephalin, and sphingomyelin, the three phosphatides which occur in animal organs, and the first two of which occur in seeds, were entirely lacking, and were replaced by a new phosphatide, calcium phosphatide, which is the calcium salt of a diglyceridephosphoric acid of the type (R_1 and R_2 representing fatty acid residues),



which has been named phosphatidic acid. The fatty acid components of this phosphatide were highly unsaturated, having an iodine value of 143. The fatty acid components of the glycerides were also very highly unsaturated, and had an iodine value in all cases examined of over 200. Such high degree of unsaturation is not found in the reserve fats of seeds, and is comparable only with the very highly unsaturated acids of the liver.

The relationship that this new phosphatide bears to the glycerides and lecithin is as follows:



It would appear that in the leaf the synthesis of phosphatides stops at the diglyceridephosphoric acid stage, and that the formation of an ester with choline to give lecithin, or with aminoethyl alcohol to give kephalin does not take place. Unlike these two latter phosphatides, calcium phosphatide is not an ester but a true salt, and the free acid or other metallic salts can be readily prepared. Calcium and magnesium phosphatide, both of which have been found in leaves, are soluble in ether and insoluble in water; consequently they must be present in the fat phase of the living cells. The sodium and potassium salts of phosphatidic acid, however, are soluble in water and insoluble in ether; consequently if they exist in the plant—and this possibility is under investigation—they must be in the aqueous phase. One of the functions of phosphatidic acid in the leaf cell, therefore, may be concerned with the permeability and antagonism of metallic ions.

The present research is an attempt to answer two questions which the unique character of the phosphatide and the presence of true glycerides in the leaf at once suggest. It is well known that if detached leaves are kept in the dark with their petioles in water there is a fall in the amount of reducing sugar and a decomposition of protein into simpler products. Both of these reactions are assumed to contribute to the leaf respiration. Is it possible that, under conditions of carbohydrate deficiency, decomposition of fatty acids can also occur? If so, are the fatty acids utilized those of the phosphatide or of the glyceride?

It has been shown by Mayer, Schaeffer, Terroine, and others (6) that in animal tissues the fatty material can be divided into two parts, an *élément variable*, which is the true reserve fat, and an *élément constant*, which is that part of the fat which cannot be diminished in amount without causing death, and which is not reduced even if death from inanition results. In other words, it is, perhaps, to be looked upon as an essential component of the animal's protoplasm. The *élément variable* includes the relatively saturated fat of adipose tissue, the *élément constant* the more unsaturated fat of organs such as the kidney, liver, or heart, all of which contain much phosphatide.

The seed fats are relatively saturated, and can undoubtedly be classed as *élément variable*, for they can be utilized for growth or respiration. The leaf fats are extremely unsaturated, and contain large amounts of phosphatides. Are they to be classified as *élément constant*? Are they simply essential components of the leaf protoplasm which will persist even if death from inanition results, or, is it possible that the phosphatide and glyceride differ from each other in this respect? If, on the one hand, calcium phosphatide is playing in the leaf metabolism the role that lecithin and kephalin play in animal metabolism, then one would expect that in extreme carbohydrate starvation the glycerides would be consumed and

the calcium phosphatide would persist. If, on the other hand, calcium phosphatide is concerned with the mobilization and transport of fatty material (the sodium and potassium salts of phosphatidic acid are soluble in water), then it may disappear during starvation, even if the glyceride persists.

An attempt has been made to throw some light on the above question by starving cut mature leaves of brussels sprout (*Brassica oleracea* v. *bullata*), which have long petioles. They were not ideal for the purpose, because in these plants the lamina growth is somewhat irregular and it is not easy to pick leaves in pairs suitable for control and experiment. They were chosen primarily because it was expected that the composition of the ether-extract would approximate to that of the cabbage given in Table I, which plant itself was not usable on account of the extreme shortness of the leaf petioles. For a true understanding of the role of phosphatides and glycerides in the plant it is essential that these two classes of substances be separated before any chemical analysis be made. Otherwise it is necessary to calculate the phosphatide content in some arbitrary way from a determination of phosphorus, and until our knowledge of the leaf phosphatides is much more extensive than it is at present, this is not chemically a sound procedure to adopt, for we do not yet know whether calcium or some other phosphatide replaces the more usual lecithin and kephelin in all leaves.

As was expected, the composition of the ether-extract of the mature leaves of the brussels sprout was very similar to that of the cabbage, and the same separation into crude phosphatide, crude hydrocarbon, unsaponifiable material and fatty acids could be readily accomplished. When the leaves were detached and starved in the dark for periods of up to eight days, no significant change in either phosphatide or glyceride was detected, even though chlorophyll had been almost completely decomposed, and the leaves were quite yellow. No general conclusion, however, can be drawn from these experiments on the fate of the fats during carbohydrate starvation, for the sugars in the lamina, contrary to what was expected, increased instead of decreasing during the period of isolation, the lamina having drawn on the sugar reserve in the fleshy petioles. It is significant that, in spite of this excess sugar, protein decomposition has gone on uninterruptedly.

II. GENERAL EXPERIMENTAL METHODS.

Methods of sampling.

The leaves for the control and starvation batches were collected in the following way. Two leaves of similar age, as shown by their position in the whorl, and of as nearly equal lamina area, were selected and cut with

petioles as long as possible. One leaf formed part of the control batch, and the other the experimental batch. The two batches of leaves were then weighed. The control leaves were analysed without delay. The ends of the petioles of the starvation batch were at once cut under water, and bunches of about twelve leaves placed in jugs of water so that the petioles were immersed. The lamina themselves were well above water and both their upper and lower surfaces were freely exposed to the air, so that respiration was not interfered with. The jugs were then placed in a dark room. The tap-water in the jugs was changed every second day, and at the same time about $\frac{1}{4}$ in. from the end of each petiole was removed under water, so as to be sure that bacterial contamination did not interfere with the free supply of water through the petiole to the leaf. At the end of four, six, or eight days as the case may be, the leaves were removed from the jugs, surface water removed from the petioles and the batch again weighed.

Preparation of water-soluble products and ether-extract.

The method was essentially that of Chibnall and Channon (3), modified in certain details.

The lamina, with or without petioles, as the case may be, were coarsely minced, mixed with an equal volume of distilled water, then finely minced twice, and finally squeezed through silk. The residue of cell material, after being treated with water, was again minced and squeezed out as before. The operation was then repeated. The residual cell-wall material was then enveloped in filter cloth, placed in a Buchner Press, and submitted to a pressure of 500 kg. per sq. cm., which was maintained until practically no more liquid was being expressed (2-3 hours). The hard cake of cellular material was then removed from the press, disintegrated in a coffee-mill, and ether-extracted for 20 hours in a Soxhlet apparatus. The combined aqueous extracts were boiled to coagulate the protein and 'fat' and then filtered. The coagulum was pressed in the same way as the cell residue, the dark-green hard mass was powdered in the coffee-mill, and the final product extracted with ether in a Soxhlet apparatus for 20 hours. The two ether-extracts were then combined and evaporated to dryness. The material thus obtained constitutes the *ether-extract*. The filtrate from the above coagulation constitutes the *water-soluble products*.

Evaluation of the values for crude protein and cell-wall material.

The ether-extracted coagulum mentioned above consists of protein and other constituents of the cell protoplasm. It contains between 10-11 per cent. of nitrogen. The cell residue also contains nitrogen. This is because a certain number of cells have not been torn open during the grinding operations; consequently their protoplasmic material has not

been dispersed into colloidal solution. During the pressing only those substances which diffuse freely through the unbroken cell-wall will have been forced out. The nitrogen in the cell residue was determined, and from it the amount of protein, assumed to have the same nitrogen content as the ether-extracted coagulum, was determined. The two combined values for protein constitute the *crude protein*. The weight of cell residue, less that of its protein content, as stated above, constitutes the *cell-wall material*.

Analysis of the Ether-extract.

The procedure was that of Chibnall and Channon (3). The ether-extracts were evaporated to dryness in a flask of the appropriate size. The material was then dried *in vacuo* to remove traces of water by immersing the flask in a boiling water bath and applying the vacuum of a water-pump. The material was then dissolved by heating with four times the weight of ether, and three volumes of acetone added. After standing a few hours in the ice-chest the precipitate was filtered off at the pump. The precipitate, which contains the phosphatide and crude hydrocarbon, was redissolved in the minimal amount of ether and reprecipitated with 2-3 volumes of acetone. The precipitate thus obtained was extracted some 6-8 times with boiling acetone, so as to remove the crude hydrocarbon. The residue insoluble in the hot acetone was dried *in vacuo* at 100° and weighed. It constitutes the *phosphatide*. That this material is similar in composition to that found in the cabbage by Channon and Chibnall, and consists chiefly of calcium phosphatide, mixed with a small amount of other products, chiefly calcium salts of fatty acids, is shown by the high ash content on elementary analysis. Found, C. 60.1; H. 9.4; ash 19 per cent. A typical analysis from the cabbage was C. 57.1; H. 8.7; ash 19.5 per cent.

The hot acetone extracts were allowed to cool slowly to room temperature, and finally in an ice-chest. The crystalline mass of crude hydrocarbon was then filtered off and dried *in vacuo* at 100°. The weight found constitutes the *crude hydrocarbon*. The mother-liquor from this last operation was added to the original acetone-ether filtrate, an aliquot part of which was saponified with 2 to 3 per cent. alcoholic potash. The unsaponifiable material (which includes the phytol of the chlorophyll) was extracted in the usual way; the soap solution, after acidification, was extracted with ether to remove fatty acids, together with the acidic products of chlorophyll decomposition, and the ether-extract evaporated to dryness *in vacuo*. The dried mass of acidic products was then extracted with light petroleum (B.P. below 40°), which removes only the fatty acids. Iodine values on the unsaponifiable material and on the fatty acids were

determined by the method of Dam (4); the sterol content of the unsaponifiable material was determined by the digitonin method of Windaus (14).

The leaf pigments were determined in an aliquot of the original ether extract by the methods of Willstatter and Stoll (13). On account of the removal of part of the magnesium from the chlorophyll during the heating of the aqueous extract of the leaves to coagulate the protein and 'fat', the determination of total chlorophyll ($a+b$) has to be modified in the following way:

The ether-extract is shaken with an aqueous solution of oxalic acid to remove magnesium, and so convert the chlorophyll to phaeophytin. The resulting solution was then submitted to Willstatter's treatment with methyl alcoholic potash, and an aqueous solution of the complex potassium salts prepared. On account of the presence of potassium soaps in this solution—due to free fatty acids in the original ether-extract—it was acidified by adding one half-volume of concentrated hydrochloric acid and filtering off the precipitated fatty acids. A comparison of the colour of this acidic solution with that of a known solution of chlorophyll treated in a similar way enabled the chlorophyll content of the original ether-extract to be calculated.

Distribution of Nitrogen and Carbohydrates in the Water-soluble Products.

The total N in solution was determined by the reduced iron method of Pucher et alii (9), whereby any nitrate N in the extract is first reduced to ammonia.

Ammonia N, amide N, and amino N were determined by the methods previously employed by Chibnall (1). Peptide N represents the increase of amino N after hydrolysis with 20 per cent. HCl for 16 hours.

Nitrate N was determined by the method of Vickery and Pucher (9).

Reducing sugar was determined by the method of MacLean (7).

Total sugar was determined by the above method after mild hydrolysis with 0.1 N HCl for 3 hours.

III. DESCRIPTION OF EXPERIMENTS.

Experiment I. Leaves picked on August 11, 1930, at 7 a.m. The control sample weighed 5,874 grm., made up of 4,172 grm. lamina and 1,702 grm. petioles. The sample for starvation weighed initially 5,732 grm. The leaves were kept in the dark room at a temperature averaging 17° for 4 days, at the end of which time they all appeared fresh and healthy, showing no signs of chlorophyll degeneration. The leaves weighed 6,185 grm., including the weight of the petiole-ends cut during the course of the experiment. There had, therefore, been a gain in weight of 453 grm. The weight of the lamina was 4,256 grm. and of the petioles 1,929 grm. For the reasons given later it is assumed that the petioles have increased

in weight proportionately to the lamina and the initial weight of the lamina before starvation was taken as 3,942 gm.

Experiment II. Leaves picked August 26, 1930, at 7 a.m. The control sample weighed 5,334 gm., made up of 3,745 gm. lamina and 1,589 gm. petioles. The initial weight of the sample for starvation was 5,274 gm. The leaves were kept in the dark room at 20°–23° for 6 days, at the end of which period most of the leaves were yellowish, and showed sign of chlorophyll degeneration. 412 gm. of leaves were yellow-brown and somewhat flaccid. These were *rejected*. The total increase in weight of the starved leaves was 632 gm., and from the relative weights of the lamina and petioles it was computed that the initial weight, before starvation, of the lamina taken subsequently for analysis was 3,280 gm.

Experiment III. Leaves picked September 26, 1930, at 9 a.m. The control sample of leaves weighed 5,642 gm. The initial weight of the sample for starvation weighed 5,561 gm. The leaves were kept in the dark room at 20° for 8 days, at the end of which period all the lamina were yellow, and showed signs of intense chlorophyll degeneration. All were still turgid, however, and none were rejected. At the end of the period of starvation the leaves weighed 6,015 gm., or, with 453 gm. representing the combined weight of the cut petiole ends, 6,468 gm., showing a gain in weight of 907 gm. The weight of the starved leaves, taken for subsequent analysis, was assumed to be 5,108 gm. (5,561 gm.–453 gm.).

IV. DISCUSSION OF RESULTS.

When the series of experiments was planned it was realized that the rather irregular development of the leaves would make comparison between the control and starvation batches somewhat difficult. But it was expected that a comparison, close enough for the limits within which the ether extract analysis can be carried out, would be obtained on a basis of fresh weight or total nitrogen—dry weight, of course, being ruled out by losses due to respiration. These expectations were not realized; the leaves during starvation took up considerable amounts of water, and there were variations in the distribution of the nitrogen between the lamina and petioles. In the case of Experiments I and II the petioles were removed before analysis, and it is necessary to know the weight at the time of picking of the lamina alone. This has been calculated from the initial and final weights of the leaves on the assumption that the petioles have increased in weight proportionately with the lamina. This assumption is probably not strictly justifiable, but proof that the initial fresh weight of the lamina calculated on this basis is accurate within the limits of the experimental error of the whole procedure of analysis is shown by the very close agreement obtained for the weight of cell-wall material in the control and starvation batches

(Table III). In Experiment III the petioles were not detached before analysis, so that no assumption of this kind has to be made, and it will be observed that the values for cell-wall material are in no better agreement than those quoted for Experiments I and II.

TABLE II.
(*Mature Leaves of Brussels Sprout.*)
The Distribution of Solids.

Experiment.	I.		II.		III.	
Period of starvation in days.	4.		6.		8.	
	Control. gm.	Starved. gm.	Control. gm.	Starved. gm.	Control. gm.	Starved. gm.
Fresh weight of lamina (in batch III with attached petioles)	4174	3942	3745	3280	5642	5108
Crude protein { Extracted	63.9	43.1	65.0	29.2	64.0	27.0
{ In residue	64.9	44.8	60.8	39.6	65.2	40.3
Cell-wall material . . .	185.1	175.2	190.5	159.5	234.6	205.3
Aqueous extract	215.6	215.1	220.3	202.3	282.1	254.9
Ether extract	21.8	23.3	21.3	17.3	23.2	18.6
Total dry weight . . .	551.3	502.4	557.9	447.9	669.1	546.1
Total sugar	48.6	87.2	42.4	45.1	119.3	84.4

TABLE III.
(*Mature Leaves of Brussels Sprout.*)

(Weight of various constituents in gm. per kg. of fresh leaves.)

Experiment.	I.		II.		III.	
Period of starvation in days.	4.		6.		8.	
	Control.	Starved.	Control.	Starved.	Control.	Starved.
Crude protein	30.9	22.3	33.6	21.0	22.9	13.2
Cell-wall material . . .	44.3	44.4	50.9	48.6	41.6	40.2
Aqueous extract	51.7	54.5	58.8	61.6	50.0	49.9
Ether extract	5.2	5.9	5.7	5.3	4.1	3.6
Total weight	132.0	127.2	149.0	136.6	118.5	106.9
Total sugar	11.7	22.1	11.3	13.7	21.1	16.5

Sugars. Table II gives the details of the preliminary analysis of the batches of leaves, and Table III the same data expressed in weights per 1 kg. of fresh leaf material. It will be seen from the latter table that the aqueous extract in Experiments I and II has increased during the starvation, and what is, at first sight, perhaps more surprising, the sugar content has increased also. That respiration has been active is shown by the loss in

weight of total solids. The explanation of the increased sugar during what can be described as a starvation period must be due to the translocation of sugar from the petiole to the lamina. At the time these two experiments were made this occurrence had not been expected, so that the detached petioles were not analysed. But that leaf petioles contain relatively very much more sugar than the lamina themselves, and that this can decrease on starvation, is brought out quite clearly in some experiments of Spoehr & McGee (11, p. 40). In the present case the weight of the petiole relative to the lamina was unusually large, and the amount of sugar in the petioles was obviously but little less than that in the lamina themselves. The leaf constituents which have been metabolized for respiration will be discussed more fully a little later, but we wish to emphasize here the fact that in Experiment III less than one-half of the lost weight can be ascribed to the sugars, and that these are still present in large amounts (15 per cent. of the dry weight). We are therefore forced to the conclusion that although the leaves had been kept in the dark for eight days, and showed complete chlorophyll degeneration, there was no deficiency of carbohydrate, and that therefore the question of carbohydrate starvation in these experiments does not arise.

TABLE IV.

(Mature Leaves of Brussels Sprout.)

Analysis of the Ether Extract.

(Weight in grm. per kg. of fresh leaves.)

Experiment.	I.		II.		III.	
	Control.	Starved.	Control.	Starved.	Control.	Starved.
Phosphatide . . .	0.89	1.02	0.68	0.82	0.57	0.42
Crude hydrocarbon . .	1.10	1.51	1.56	1.70	1.24	1.42
Unsaponifiable material .	1.23	1.40	1.35	1.12	0.84	0.90
Fatty acids . . .	1.00	1.07	0.94	0.95	0.58	0.57
Chlorophyll . . .	0.96	0.94	0.96	0.25	0.52	0.12
Total ether extract . .	5.23	5.91	5.66	5.27	4.11	3.67
Sterols			0.12	0.10	0.11	0.10
Total ether extract less (chlorophyl-phytol) .	4.58	5.29	5.02	5.10	3.74	3.55

Ether-extract. Details of the analyses are given in Table IV. In comparing the individual fractions it is necessary to remember that some constituents are extracted by the ether more readily than others. The experience gained in this laboratory during this summer when analysing the ether-soluble material of some 40 samples of various grasses has shown that the crude hydrocarbon is sometimes difficult to extract completely. If the leaves are simply dried and then extracted with ether, the amount of this fraction obtained is generally less than that obtained from

the fresh leaves by the methods outlined in this paper. The reason for this is not yet clearly understood. In the case of fresh leaves the variations in yield seem to be in some way connected with the ease with which the leaf material is disintegrated in the mincing machine. The cell-wall material of the starved leaves seemed to be less 'rigid' than that of the control leaves, and was much more easily minced; it is to this observation that we prefer to ascribe at present the somewhat higher yields of crude hydrocarbon obtained from the starved leaves, and not to any possible synthesis. The variations in the tables, therefore, of this fraction are considered, at the present time, to be non-significant.

Again, the quantitative separation of the crude phosphatide is not easily reproducible within narrow limits. It is precipitated from the ether extract together with the crude hydrocarbon by acetone, and is separated from the latter by repeated extraction with hot acetone. The solubility of the crude phosphatide in this hot solvent is very much less than that of the crude hydrocarbon, but even so it has a small solubility. Consequently, in the separation of the two constituents the volume of hot acetone that has to be employed can readily influence the final yield of crude phosphatide. Experience does not suggest that the variations shown in the table are significant.

The glycerides are readily extracted by the ether, and the values for fatty acids given in the table show quite clearly that there has been no loss during the starvation period. The iodine value of the fatty acids (130–140) showed no significant change.

So little is known about the constituents of the unsaponifiable material that it does not seem worth while to discuss this fraction in any detail. The sterol content shows no significant change, nor do the carotinoids (carotene and xanthophyll).

TABLE V.
(*Mature Leaves of Brussels Sprout.*)
Distribution of Fats, &c.

(In percentages of total ether-extract less (chlorophyll-phytol).)						
Experiment.	I.		II.		III.	
	Control.	Starved.	Control.	Starved.	Control.	Starved.
Phosphatide	19.0	19.3	13.6	16.0	15.2	11.8
Crude hydrocarbon . .	23.9	28.5	31.0	33.4	33.2	40.0
Unsaponifiable material .	26.8	26.6	26.8	22.0	22.5	25.3
Fatty acids	21.8	20.3	18.7	18.6	15.4	16.0

Within the limits of experimental error, then, the figure given in Table IV shows that there is no significant change in the fat (glyceride plus phosphatide) of leaves which have been kept in the dark until chlorophyll degeneration is nearly complete, and the leaves are on the point of

death. The point is perhaps brought out more markedly in Table V, in which the components are set out in terms of ether-extract less (chlorophyll-phytol), (it is assumed that in the decomposition of the chlorophyll, the phytol is at once hydrolysed off, and, remaining in the fat phase of the chloroplasts, is still present in the unsaponifiable material). This method of comparison is, of course, independent of the value assigned to the fresh weight of the leaves taken for experiment.

TABLE VI.

(Mature Leaves of Brussels Sprout.)
Showing the Distribution of Nitrogen.
(Weight in grm. per kg. of fresh leaves.)

Experiment.	I.		II.		III.	
	Control.	Starved.	Control.	Starved.	Control.	Starved.
Total N	4.10	4.46	5.21	4.69	3.83	3.92
Crude protein N	3.18	2.60	3.85	2.16	2.71	1.42
Water-soluble N	0.92	1.86	1.36	2.53	1.12	2.50
Ammonia N	0.07	0.16	0.11	0.27	0.08	0.16
Amide N	0.15	0.24	0.20	0.35	0.17	0.56
Nitrate N	0.06	0.05	0.04	0.00	0.10	0.13
Amino N					0.24	0.97
Peptide N					0.09	0.00

Protein metabolism. One unlooked-for result of the present research has been the clear demonstration that when detached leaves are kept with their petioles in water protein decomposition can, in certain cases, take place as readily in the presence of abundant carbohydrates as it does during carbohydrate starvation. Deleano (5) showed that in the case of vine leaves there was no appreciable break-down of protein until the sugars were much depleted. This seemed to confirm the older ideas of Pfeffer and Prianischnikoff that break-down of protein was caused by a deficiency of carbohydrate. Chibnall (2) and Mothes (8) have since shown in these circumstances that protein break-down goes on nearly as readily when the detached leaves are kept in the light as in the dark. In both these cases, however, the sugar in the leaf was not determined, so that the deficiency or otherwise of carbohydrate has had to be assumed. Spoehr and McGee (11) showed that in the case of excised leaves of *Helianthus* fed with glucose there was a threefold increase of amino N, although the sugar level of 7 per cent. of dry weight was maintained. The data given in Table VI show quite clearly that protein decomposition, with the production of large amounts of acid amides, goes on even if the sugar content increases to over 20 per cent. of the dry weight of the leaf. The data also suggest that translocation of excess soluble nitrogen from the lamina back to the petiole has occurred in Experiment II. We do not propose at present to discuss more fully the bearing that these observations have on theories of protein metabolism in leaves.

V. SUMMARY.

1. A short account is given of the fats which occur in leaves, and the changes that these may undergo during starvation if their metabolism is analogous to that observed in the animal body.

2. Experiments have been made with cut mature leaves of brussels sprout, which have been kept with their petioles in water in the dark for varying periods.

3. The fleshy petioles have supplied sugar to the lamina during these periods, and there has been no carbohydrate deficiency.

4. There was no significant change in the constituents of the fat, even although at the end of eight days in the dark the chlorophyll had almost completely disappeared.

5. Brief reference is made to the fact that the leaves kept in the dark show a continuous break-down of protein, in spite of the presence of abundant carbohydrate.

Our thanks are due to Mr. Hales of the Chelsea Physic Gardens for the care bestowed on the plants used, and to Mr. R. G. Westall for nitrogen and sugar analyses.

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Development of the Embryo-sac in *Calotropis procera* with Especial Reference to Endosperm Formation.

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With thirty-six Figures in the Text.

INTRODUCTION.

HOFMEISTER (18) described a spherical embryo-sac in *Cynanchum nigrum*. In *Stapelia* and *Asclepias* he records the presence of an elliptical embryo-sac, which is surrounded from the beginning by a single layer of cells. This layer is destroyed long before fertilization takes place. The developing egg-cell forms a long cellular thread. After the formation of the embryo, the proximal end of the cellular thread grows backwards and often penetrates the embryo-sac wall.

Vesque (38), working on *Vinca minor*, found that the embryo-sac mother-cell is covered by a one-layered nucellus, and mentions that the development which takes place in the Asclepiadaceae is similar to that of the Apocynaceae.

According to Chauveaud (2) the nucellus of the species of *Vincetoxicum* which he investigated consists of one cell only, namely, the embryo-sac mother-cell. He denies the presence of an integument. The antipodes may be lying close to each other, or with one slightly higher than the rest. These structures disappear immediately after fertilization.

The same author believes that the embryo-sac development follows the *Lilium*-type and that fertilization of the synergids, when it occurs, takes place by extra nuclei in the pollen tube. In *V. medium* it is rare to find one embryo only, and as many as five embryos have been described.

Hubert (20) found a straight embryo-sac in *S. planifolia*, tapering towards the antipodal end. The antipodes lie in a row.

Billings (1) found that in *V. officinale* the integument grows rapidly in thickness after fertilization, mainly by the division of the cells, but also by

their enlargement. In the beginning the growth of the integument is more rapid than that of the embryo-sac.

The fertilized egg-cell starts dividing before the endosperm is well developed.

Frye (10) studied some species of *Asclepias*, mainly *A. cornuti*. He contradicts Chauveaud's statement concerning the nucellus and integument. He found that occasionally two or three archesporial cells are present side by side, but in no case was more than one found to develop further in the same ovule. A single integument develops around the rudimentary nucellus, so that the nucellus becomes deeply inserted in the tissue. The nucellus soon degenerates and is replaced by the embryo-sac. The dyad cells (the half of a tetrad group) do not develop simultaneously, the micropylar one often dividing later in *A. tuberosa*. Any one of the tetrad cells is capable of giving rise to an embryo-sac, even the uppermost one. In most cases it is the lowermost megaspore which develops, and the other three disappear very quickly. Occasionally more than three antipodes have been found, and once the presence of fewer than three has been recorded. Very soon the embryo-sac destroys the nucellus and much of the surrounding tissue. In one instance the embryo-sac reached the surface of the ovule, and in many cases it reached close to the surface. Sometimes one of the synergids is destroyed. The polar nuclei unite whether fertilization has taken place or not.

No division of the egg-cell takes place before the endosperm has passed the sixteen-nucleus stage.

After fertilization, the ovule starts active growth. At the time of the third division of the endosperm the epidermis at the micropylar end gets intensely activated and forms a pappus. Each hair consists of one cell and one nucleus.

Dop (5) observed that in *A. Douglassi* the uppermost wall of the tetrads is oblique, and the lower cell develops into an embryo-sac while the three upper ones degenerate.

He mentions that there is no integument in *Asclepias*, *Gomphocarpus*, *Marsdenia*, *Ceropegia*, *Stapelia*, and *Arauja*.

Seefeldner (29), examining *C. vincetoxicum*, confirms Chauveaud's statement concerning the absence of integument. He mentions that the upper dyad cell is not developed, but is absorbed, and the lower dyad develops into an embryo-sac (Scilla-type). The antipodes lie in a depression in the embryo-sac. They have very small nuclei, and are indistinct and disappear totally during later stages.

For sometime the egg-cell and the embryo-sac remain almost unchanged, while the endosperm is more advanced in development. In the meantime the ovule increases in size to a great extent. The fertilized egg divides and gives rise to a cell-complex carrying polyembryos.

Guignard(14) found that the nucellus consists of an archesporium covered with a rudimentary epidermis. He mentions that in *A. curassavica* the archesporium becomes embedded in the ovule and occupies approximately the centre. Later in development the embryo-sac reaches the surface of the ovule. The polar nuclei do not seem to fuse before fertilization. The four megaspores are superposed, though exceptionally the upper two lie side by side. In *Marsdenia* the lower dyad seems to divide somewhat later than the top one. The upper megaspore may be the earliest to degenerate, and for a time only three sister cells can be seen. In *Arauja sericifera* several archesporia have been found in the same ovule, but one only continued its development.

Guignard in a later paper (15) describes the occurrence of polyembryony in Asclepiadaceae (*Vincetoxicum*).

Francini (9), who investigated *S. asterias* and other species of *Stapelia*, finds that the lowermost megaspore develops into an embryo-sac. The antipodes soon show signs of degeneration, and according to his figure 8 they lie in a uniseriate row (*S. asterias*). He records the presence of one integument. In another paper (1927) he mentions that in *Cynanchum acutum* the two upper megaspores lie side by side.

He describes the presence of polyembryos in *Vincetoxicum speciosum* (8).

The Endosperm.

Hofmeister (18) describes the endosperm formation in Asclepiadaceae and mentions that in *C. nigrum* and *A. syriaca* a number of free nuclei occur after fertilization and form the endosperm. In *A. syriaca* wall-formation takes place before the egg divides, so that the embryo-sac is quite filled with endosperm.

Chauveaud (2) states that in *Vincetoxicum* the walls of the endosperm do not appear until the eight-nucleus stage.

Frye (10) mentions that in *A. cornuti* the first division of the endosperm nucleus occurs soon after fertilization. The second and third divisions rapidly follow. Up to this time the nuclei divide simultaneously and no walls are formed. In the eight-nucleus stage of the endosperm the formation of the walls by indentation begins. The division into sixteen nuclei seems to be nearly simultaneous. By this time the cells have become somewhat walled off, and the divisions, from this time on, are not simultaneous.

Seefeldner (29), studying *C. vincetoxicum*, states that free nuclear division of the endosperm occurs. The nuclei lie near the walls of the embryo-sac. Later they become approximated and cell walls begin to appear.

According to Francini (8), in *C. acutum* the walls of the endosperm develop while the number of the nuclei is still very small.

Previous Work on Calotropis.

In the available literature, Guignard (14) is the only author who refers to the embryonic development of *Calotropis*, and this reference is purely casual.

C. procera is an Asclepiad shrub or tree known locally as 'oshar' and reaches as far north, in Egypt, as the Fayum.

It contains a poisonous latex which is said to cause blindness if put in the eye. The fine hairs of the seeds are used as a stuffing.

Material and Methods.

Most of the early stages of *C. procera* (Ait) were collected in January from Kharga Oasis. More advanced stages were brought in May from the Orman Gardens, Giza. *Daemia tomentosa* (Pomel.) was collected in April from the eastern desert, a few miles east of Heliopolis, and also from Wadi Hof, north-east of Helwan. *Kanahia glaberrima* N.E.Br. was taken in June from the Zohria Gardens, Gezira.

Unless otherwise stated, the description given below refers to *Calotropis*. The other material was used only for comparative purposes.

The fixative which gave best results was Zenker's; Karpechenoe's and Carnoy's fluids were also used. The young buds were put as a whole in the fixing solutions. Flowers were dissected, and only the carpels were fixed. The fruits were cut into small pieces, each containing a part of the endocarp and the interior structures. Chloroform was used as a clearing agent and the material was embedded in paraffin in the usual way. Sections were cut 5–10 μ thick. Heidenhain's iron-alum-haematoxylin was used as a stain and light green in clove oil as a counterstain.

All figures (except 33–6) were sketched with the aid of an Abbé camera lucida with a Zeiss $\frac{1}{2}$ oil immersion objective.

Differentiation of the Archesprial Cell.

The ovary of *C. procera* has two carpels with two large axile placentas carrying numerous ovules. The number of ovules in *D. tomentosa* is much less than that in *Calotropis*.

The ovules bulge out as hemispherical bodies (Fig. 1) and are formed in rows, the lowermost ovule in each row being the oldest. During the later stages of development they become more or less obliquely orientated.

A hypodermal cell is recognized by its larger nucleus at the upper outer angle of the ovule and forms the archesporium (Fig. 2). This archesporium becomes quite conspicuous before the funicle is distinctly differentiated. Although in this material only one archesporial cell has

been observed to each ovule, yet later stages show that in some ovules more than one may occur (Figs. 21-3).

This agrees with what has been observed by Frye (10) in *Asclepias*, and Guignard (14) in *Arauja*.

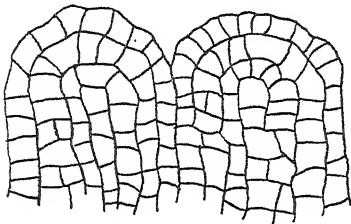


FIG. 1.

FIG. 1. *Calotropis procera*.

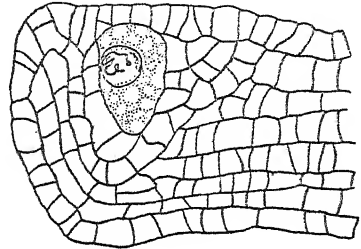


FIG. 2.

Two young ovules. $\times 500$.
Embryo-sac mother-cell. $\times 500$.

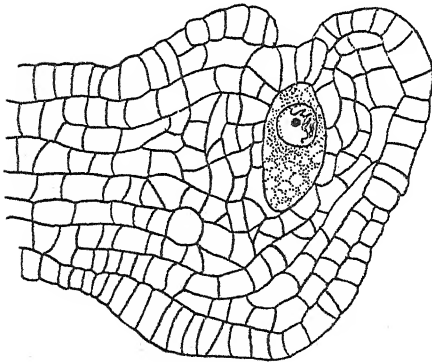


FIG. 3.

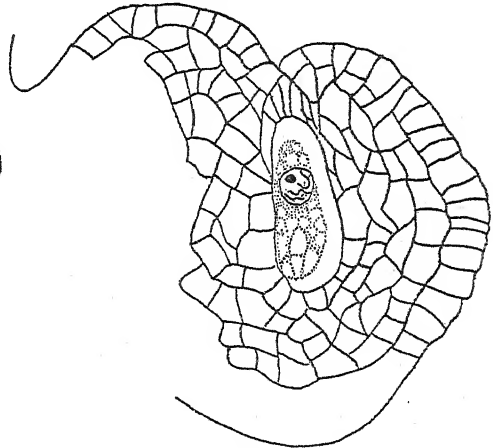


FIG. 4.

FIGS. 3-4. *Calotropis procera*. 3. Development of the integument. $\times 500$.
4. More advanced stage. $\times 500$.

The Development of the Integument.

The archesporial cell is covered above by a small 2- or 3-celled group of epidermal cells arranged radially, which are not, however, sharply differentiated from the neighbouring epidermal cells (Fig. 2). This nucellar cap of cells grows for a short time and is covered by an integument which surrounds the top of the archesporial cell (Figs. 3-4). There is one integument, as Frye (10), Guignard (14), and others have described. The ovule is never naked as stated by Chauveaud (2) in *Vincetoxicum spp.*, Dop (5) in *Asclepias*, *Gomphocarpus*, *Marsdenia*, *Arauja*, &c., and Seefeldner (29) in *C. vincetoxicum*. Dop's figures, nevertheless, show the presence of an integument, though the interpretation appears at fault.

The nucellus consists of the archesporial cell and the cap of epidermal cells covering it (Figs. 3-7). Later, the archespore becomes embedded in the ovule, and the epidermal cap disappears during the 2- to 4-nuclear stage of the embryo-sac.

The Formation of the Tetrad.

By two successive divisions the tetrad stage is attained and the cells are superimposed in *Calotropis*, *Kanahia*, and *Daemia* (Figs. 7-9). In one case the basal dyad probably divided before the top one (Fig. 8). The tetrad is formed in the normal way. This differs from the Scilla-type as Seefeldner (29) believes in *Cynanchum*, and from the Liliun-type according to Chauveaud's (2) investigations in *Vincetoxicum*. The uppermost wall of the tetrad is often oblique (Fig. 9), as Dop (5) reports for *A. Douglasi*. The basal megaspore usually forms the embryo-sac, and the three upper cells rapidly degenerate. From the study of the cases of disintegration it seems that degeneration begins from the micropylar region working downwards. Later stages show that more than one megaspore may start development (Figs. 19, 20), but in no case have two completely developed embryo-sacs been observed. Frye (10) in *Asclepias*, and Francini (14) in *Cynanchum*, have observed that one of the other megaspores may develop instead of the lowermost.

No tapetum has been observed. The number of chromosomes could not be determined for certain, but it seems to be small. Gaiser (12) records in his list of chromosome numbers in angiosperms that Strasburger (33) found ten chromosomes in *A. cornuti*, while W. C. Stevens (32), Gager (11), and Finn (7) report the presence of twelve chromosomes in the same species.

Formation of the 8-nucleate Embryo-sac.

The embryo-sac rapidly destroys the nucellus and some of the surrounding tissue and becomes deeply seated in the ovule. It becomes slightly curved (Figs. 12-16) and the chalazal end tapers in *Kanahia* (Fig. 15). In *Daemia* the embryo-sac is elongated, curved, and more or less narrow at both ends (Fig. 16).

The embryo-sac passes rapidly through the 2-, 4-, and 8-nucleate stages (Figs. 10-14). The synergids are pear-shaped, with their beaks directed towards the micropyle (Figs. 14-16). The twopolar nuclei approach each other and fuse before fertilization (Figs. 14-15). Frye (10) in *Asclepias* reports that the polars have usually not fused when the tube bursts.

The antipodes are small and lie near each other in *Calotropis*, but they are superimposed, forming a row of cells in *Kanahia*, and probably in *Daemia* too (Figs. 14-16). Hubert (20) and Francini (8) have observed that the antipodes in *Stapelia* lie one on the top of the other in a row. The antipodes last for a short time only, disappearing rapidly after fertilization ;

in fact, there are signs of degeneration just before fertilization. The short life of antipodes has been observed by nearly all investigators. Frye (10) only reports and figures remains of the antipodes in the thirty-two nucleate

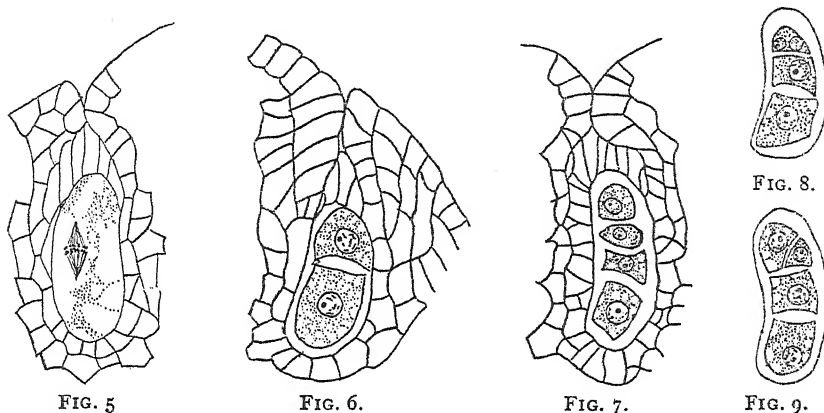


FIG. 5.

FIG. 6.

FIG. 7.

FIG. 8.

FIG. 9.

FIG. 5. *Calotropis procera*. Reduction division of the mother-cell. $\times 500$.

FIG. 6. *Calotropis procera*. Dyad stage. $\times 500$.

FIGS. 7-9. *Calotropis procera*. 7. Tetrad with superimposed cells. $\times 500$. 8. Basal dyad dividing before the top cells. $\times 500$. 9. Tetrad with oblique uppermost wall. $\times 500$.

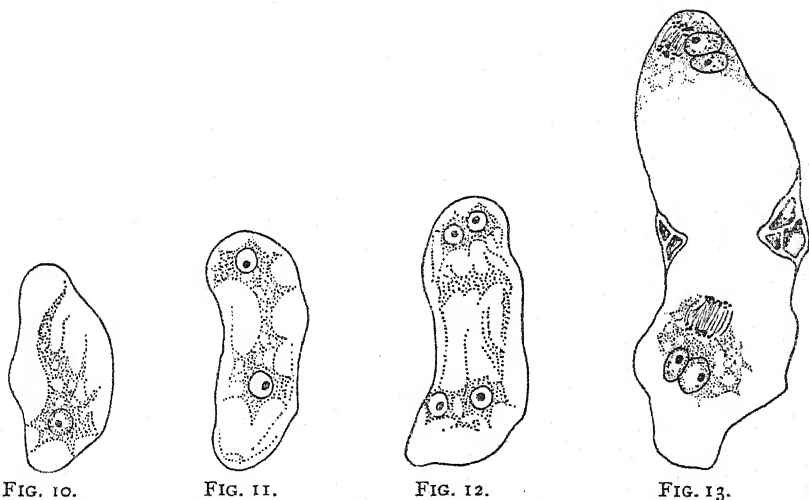


FIG. 10.

FIG. 11.

FIG. 12.

FIG. 13.

FIGS. 10-13. *Calotropis procera*. Embryo-sac. 10. Uninucleate. $\times 500$. 11. Binucleate. $\times 500$. 12. 4-nucleate stage. $\times 500$. 13. 8-nucleate stage. $\times 835$.

stage of the endosperm. He also found a few tracheides in the ovule near the antipodals, which is rather exceptional. The synergids persist longer.

In no case have I found that the embryo-sac reaches the surface of the ovule, or even shifts towards it, as reported by Frye (10) for *Asclepias*, and Guignard (14) for *Vaccinium officinale* and *S. variegata*.

Abnormalities.

Many cases of abnormalities are found in *Calotropis* and *Kanahia*. Thus, Fig. 17 shows an irregular case, where two groups of cells arranged just like pollen-tetrads are found in one and maybe in two embryo-sacs.

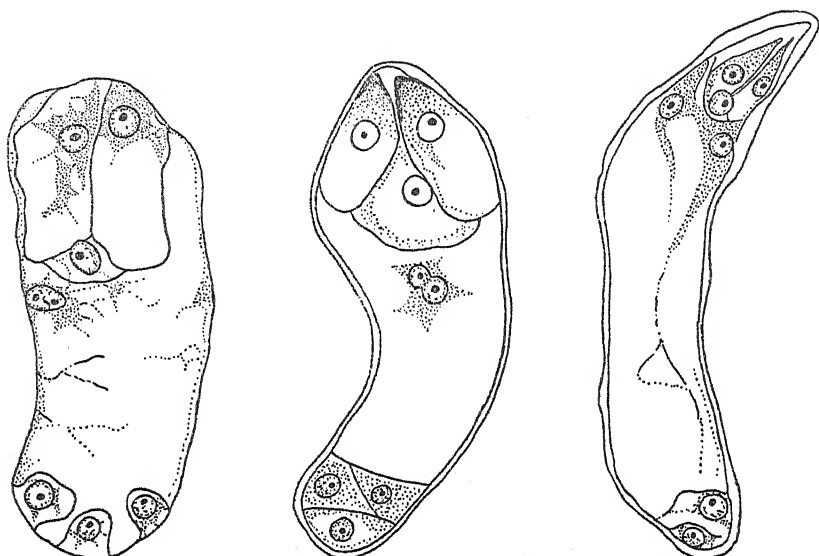


FIG. 14.

FIG. 15.

FIG. 16.

FIG. 14. *Calotropis procera*. Embryo-sac just before fertilization. $\times 835$.

FIG. 15. *Kanahia glaberrima*. Embryo-sac tapering towards the chalazal end. $\times 835$.

FIG. 16. *Daemia tomentosa*. Embryo-sac tapering at both ends. $\times 835$.

Masters (24) has found in *Rosa arvensis* an anomaly, where the ovary contained both ovules and anthers. The opposite type of anomaly (an ovule situated in an anther) is described by Täckholm (37) in a garden rose.

An abnormal 8-nucleus stage can be seen in Fig. 18. About the centre of the upper half of the embryo-sac six nuclei are irregularly disposed, while about the centre of the lower half two nuclei can be recognized. Such cases of non-polarity are not infrequent.

A different type of abnormality is illustrated in *Calotropis* and in *Kanahia* in Figs. 19–22 and 23, respectively. In all these the ovule contains more than one embryo-sac in different stages of development. Such additional embryo-sacs may arise, either from extra archesporial cells (Figs. 21–23) or from the development of more than one megaspore (Figs. 19, 20).

The Fertilized Ovule.

In the fertilized ovule the antipodes always disappear (Figs. 24, 25). Occasionally one of the synergids is destroyed, as reported by Frye (10)

in *Asclepias*. Chauveaud (2) believes that in *Vincetoxicum* the fertilization of the synergids takes place by extra nuclei from the pollen-tube. Such an occurrence has not been observed in *Calotropis*.

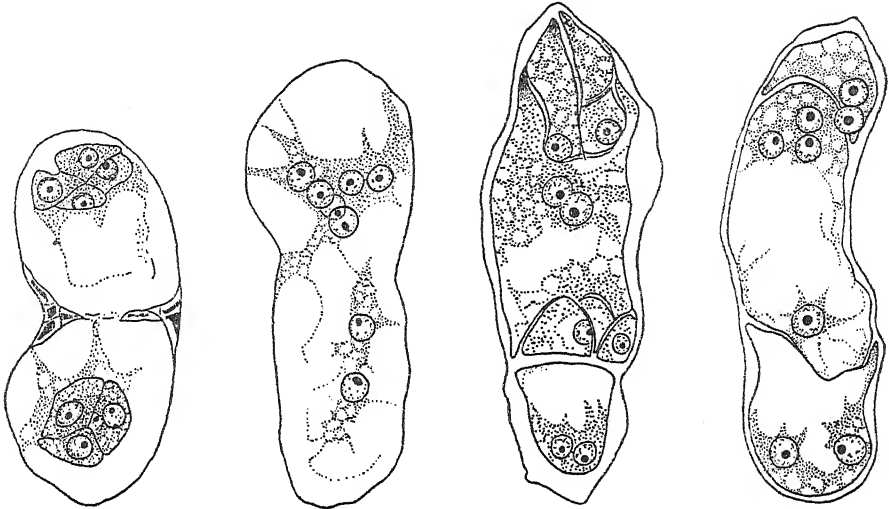


FIG. 17.

FIG. 18.

FIG. 19.

FIG. 20.

FIGS. 17-20. *Calotropis procera*. Abnormal embryo-sacs. 17. Two pollen-like tetrads enclosed in one (or two) embryo-sacs. $\times 835$. 18. Irregular 8-nucleate stage. $\times 835$. 19. Two embryo-sacs developed from two megaspores. $\times 835$. 20. Three megaspores developing. $\times 835$.

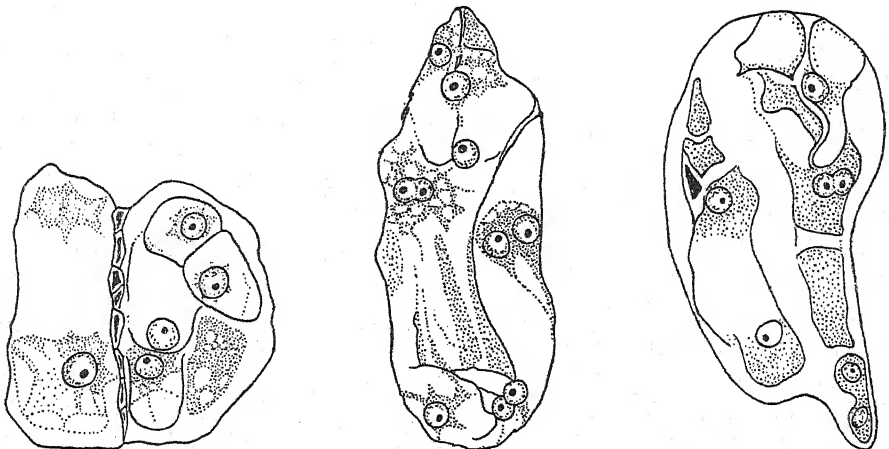


FIG. 21.

FIG. 22.

FIG. 23.

FIGS. 21-23. Two archesporial cells developing. 21. *Calotropis procera*. One embryo-sac, uninucleate, the other 4-nucleate. $\times 835$. 22. *Calotropis procera*. One embryo-sac binucleate and the other more advanced. $\times 835$. 23. *Kanahia glaberrima*. Two developed embryo-sacs. $\times 835$.

The Endosperm.

In its early stages endosperm develops by free nuclear division (Figs. 24, 25). Walls first appear at the 16-nucleus stage (Fig. 26). They seem to be formed by indentation from the periphery. Unfortunately,

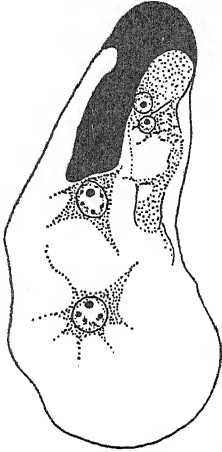


FIG. 24.

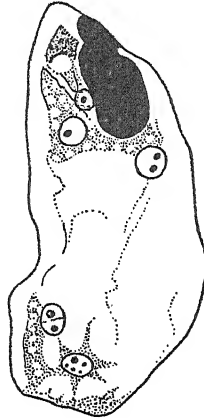


FIG. 25.

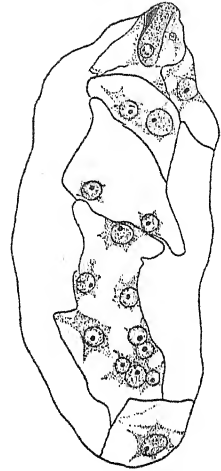


FIG. 26.

FIGS. 24-25. *Calotropis procera*. After fertilization. 24. Egg-cell, remaining synergid, and the two first free endosperm nuclei. $\times 835$. 25. After the second division of the endosperm nucleus. $\times 835$.

FIG. 26. *Calotropis procera*. After the fourth division of the endosperm nucleus some walls are visible. $\times 557$.

no 8-nucleus stage was found, and it is impossible to say whether the wall-formation starts immediately after the third division or not. At the 16-nucleus stage wall-formation does not take place between all endosperm nuclei, for some of the cells still contain more than one nucleus.

The present study, together with the observations of previous authors on various representatives of the Asclepiadaceae (page 505), point to the fact that cell-wall formation in the endosperm of this family takes place comparatively early. An early appearance of cell-walls in the development of the endosperm has been observed also in other families; Svensson (36) gives a summary of such cases and refers to *Lycopsis* sp., *Thismia javanica* (Svensson (36)), *Rafflesia Patma* (Ernst and Schmidt (6)), *Voyria* spp. (Johow (21)), *Asclepias* (Frye (10)), Rubiaceae (Lloyd (23)), *Hamamelis virginianae* (Shoemaker (30)), *Eupatorium glandulosum* (Holmgren (19)), and *Hieracium aurantiacum* (Schnarf) (28)).

Cases of irregular development of the endosperm of *C. procera* certainly occur. Thus a cellular endosperm right from the beginning (*ab initio* cellular endosperm) is shown in Figs. 28 and 29. A clear wall separates the two first formed endosperm nuclei. Though it was not possible to

determine whether cell-walls were formed in the spindle or by indentation from the periphery, it is probable that the latter method obtains.

The occurrence of the two types of endosperm formation in the same

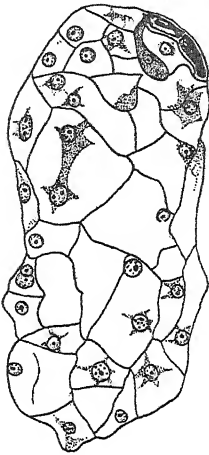


FIG. 27.

FIG. 27. *Calotropis procera*. Embryo-sac filled with endosperm cells, the egg still undivided. $\times 333$.

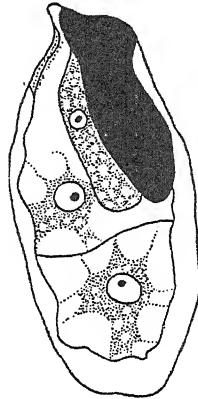


FIG. 28.

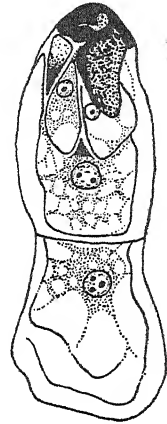


FIG. 29.

FIGS. 28-29. *Calotropis procera*. Cases of wall formation after the first division of the endosperm. $\times 835$.

family is frequent. Thus Svensson (36) has found typical nuclear and typical *ab initio* cellular endosperm types, as well as intermediate stages, in Hydrophyllaceae and Boraginaceae. Cases where the two types have been found in the same genus are, on the contrary, very rare. Svensson (36) found such a case in *Phacelia* spp. Hegelmaier (17) and Lagerberg (22) found the same in *Sambucus*. Both types of endosperm formation in the same species have been recorded by Stevens (31) in *Vaccinium corymbosum*, by Holmgren (19) in the apogamous *Eupatorium glandulosum*, and by Schnarf (28) in the apogamous *Hieracium aurantiacum*! According to the last two authors, if the polar nuclei fuse, the endosperm formation is cellular; if the fusion fails, the polar nuclei divide independently, and a short nucleate stage precedes the cellular development of endosperm. Palm (26) has found in the tobacco plant that walls are not formed when the secondary embryo-sac nucleus undergoes an amitosis-like division. Haberlandt (16) was able to induce the formation of cell-walls in *Oenothera Lamarckiana* by carefully wounding some embryo-sacs. He concluded that 'wound hormones' are responsible for such formation.

Four of the above-mentioned cases are found to occur, more or less, under unnatural conditions. *Eupatorium* and *Hieracium* are apogamous, and in such plants many abnormalities occur. In the tobacco plant there is an abnormal case of amitosis, and in *Oenothera* wall-formation is induced

only under experimental conditions. The case of *Vaccinium* (Stevens (31)) seems to be the only one recorded as occurring in a normal plant and under natural conditions. Dahlgren (4) is of the opinion that Stevens' work should be corroborated. As a matter of fact, the figure of the free nucleate endosperm is very similar to the 8-nucleate stage of the embryo-sac. Here, in the case of *C. procera*, both extreme types of endosperm formation are found even in the same ovary.

Strasburger (34) and others believe that the nuclear and cellular types of endosperm are caused by 'growth correlation'. Sussenguth (35) attributes the difference to the 'space factors of the embryo-sac' ('raumlichen Momenten des Embryosackes'). According to this author, in long, narrow embryo-sacs the endosperm would be cellular, while in the broad, short type nuclear endosperm prevails. Samuelsson (27) pointed out that such an explanation cannot apply in cases where the first wall is longitudinal.

Svensson (36), working on *Phacelia*, finds no support for Strasburger's and Sussenguth's views. He points out that the size and shape of the embryo-sacs containing cellular endosperm are similar to those of embryo-sacs containing the other type of endosperm. As we find both types included in one and the same ovary, we are in agreement with Svensson's opinion.

Dahlgren (4) is of the opinion that the nucleate type is the more primitive, and that the *ab initio* cellular endosperm must have developed quite independently in different systematic groups. Svensson (36) mentions that between the normal nuclear and the *ab initio* cellular endosperm nearly all imaginable intermediate types are found. These facts seem to support the view that the development has the tendency to go in a way which leads to an early tissue formation in the endosperm. In several cases these developmental tendencies have ended with the formation of the *ab initio* cellular endosperm type, which therefore in *Calotropis* is to be regarded as the derived type.

Some investigators lay stress upon the systematic value of the type of endosperm formation. Samuelsson (27) ranked the type of endosperm formation, from this point of view, with the number of integuments, etc. Other investigators tended to overestimate the value of the endosperm character. Thus, Dahlgren (4) has been first convinced of the endosperm type as a systematic criterion, but after his own discoveries in the Compositae (4), and Svensson's (36) in some other sympetalous families, he became less certain.

The present investigation shows clearly that nuclear and *ab initio* cellular endosperm types occur in the same family, the same genus, the same species, and even in the same ovary. It is evident, therefore, that such criterion cannot be used systematically with safe results.

The Embryo.

The fertilized egg rests for some time and does not start development until the embryo-sac is filled with endosperm cells (Fig. 27). It divides

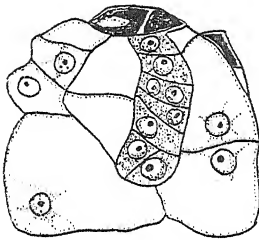


FIG. 30.



FIG. 31.

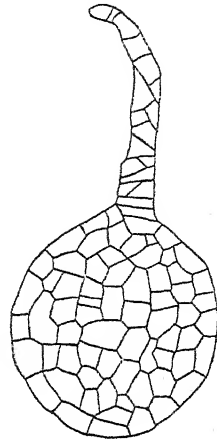


FIG. 32.

FIGS. 30-32. *Calotropis procera*. Early stages in the development of the embryo.
× 500. × 500. × 333.



FIG. 33.

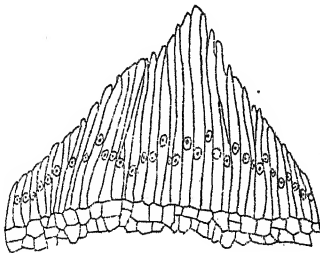


FIG. 34.

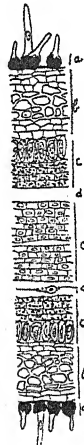


FIG. 35.

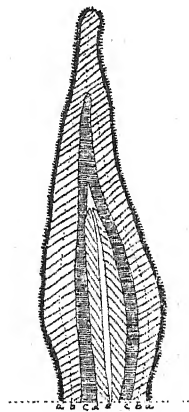


FIG. 36.

FIG. 33. *Calotropis procera*. Fertilized ovule (4-nucleate endosperm), with pappus.

FIG. 34. *Calotropis procera*. Pappus magnified.

FIG. 35. *Calotropis procera*. Transverse section of seed. (a) Epidermis with papillae. (b) Outer zone of testa. (c) Inner zone of testa. (d) Endosperm (partly absorbed). (e) The two cotyledons

FIG. 36. *Calotropis procera*. Longitudinal section of seed; lettering as Fig. 35.

transversely and gives rise to a cellular filament 'proembryo' (Fig. 30). The distal cell of the proembryo divides first longitudinally and then in all directions, giving rise to an embryo (Figs. 31 and 32). In the meantime

the suspensor increases in size. Later on in development the embryo becomes massive, and the suspensor elongates, forcing the young embryo down into the principal mass of the endosperm. No polyembryos such as were reported by Chauveaud (2), Seefeldner (29), Guignard (15), and Francini (8) in other Asclepiadaceae were recognized in *Calotropis*. Nor were haustoria detected in the material examined.

The Ovule.

The ovule increases rapidly in size and acquires an elongated form (Fig. 33). About the time of the second division of the endosperm the epidermis at the micropylar end gets activated and forms a pappus (Fig. 33). Each hair is unicellular and uninucleate (Fig. 34), as Frye (10) describes in *Asclepias*.

The epidermis of the testa has papillae (Fig. 35). Under the epidermis lie a few layers of flattened cells containing crystals. The innermost layers consist of dark, thick-walled cells, containing starch. Figs. 35 and 36 show these layers of testa enclosing the persistent endosperm and the two cotyledons of the embryo. The testa wall resembles in construction that of *Morrenia* (25).

SUMMARY.

1. A single hypodermal cell forms the archesporium.
2. There is a single integument.
3. The nucellus consists of an archesporial cell and a cap of epidermal cells covering it.
4. The tetrad is formed in the normal way.
5. No tapetum has been observed.
6. Abnormal embryo-sacs are found.
7. Additional embryo-sacs may arise in the same ovule, either from extra archesporial cells or from the development of more than one megaspore.
8. The polars fuse before fertilization.
9. Endosperm with free nucleus as well as endosperm showing a cellular structure from the beginning have been found in the same ovary.
10. The fertilized egg-cell rests until the endosperm has become cellular.
11. No polyembryony and no haustoria were observed.
12. The pappus is composed of unicellular and uninucleate epidermal hairs.
13. The testa has two distinct zones and a papillate epidermis.

I am greatly indebted to Prof. Gunnar Täckholm, under whose personal

direction most of this work has been done, for his criticisms and also for providing many references. I am also indebted to Prof. F. W. Oliver for valuable suggestions and help.

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The Pectic Substances of the Carrot, with Reference to their Decomposition by *Bacillus carotovorus*.

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THE action of many bacteria and fungi upon plant tissues, whereby softening and decay are caused, has long been recognized. Among the organisms whose effect in this direction is best known is *Bacillus carotovorus*, which is responsible for the 'soft rot' of carrots and other root vegetables. This organism was studied in considerable detail by Jones (8), (9), (10), who was able to prove that the rotting was produced by solution of the pectin of the middle lamella tissue, solution being brought about by an enzyme secreted by the bacteria. More prolonged action resulted in partial attack of the actual cell-wall material (protopectin), which, however, was never completely dissolved. The cell contents were never affected, except where mechanical rupture had occurred. Jones considered that an abundance of moisture was necessary for the attack to proceed, and further stated that the action of the organism was confined to parenchyma.

Later work by Brown (2), (3), who worked with a powerful enzyme preparation from *Botrytis cinerea*, confirmed the observations of Jones. In these experiments again the solvent action of the enzyme was confined to the pectic constituents of the middle lamella and cell-wall, the cellulose being unaffected.

In examining the action of *B. carotovorus* on the fleshy root of the carrot, it has been noted frequently that there is a marked difference in the rates at which the well-defined concentric zones (cortex and stele¹) in the root undergo rotting. Similar observations were made by Davison and Willaman (6), who observed that large and small potatoes of the same

¹ The tissue briefly described as 'cortex' consists of cortex with some phloem, the 'stele' being xylem and pith.

type, and the cortex and medulla of the same tuber, were macerated by pectic enzymes at different rates. Such differential attack in the case of the carrot was noted by Jones, who concluded that the difference was occasioned by a difference in the moisture content of the two zones, the tissue having a greater moisture content being more susceptible to attack. It was found, however, that there was little difference between the moisture content of the two zones in a number of roots examined; for instance, the moisture content of typical examples was found to be: cortex 88.8 per cent., 89.0 per cent., 90.1 per cent., 90.4 per cent.; stele, 90.5 per cent., 91.3 per cent., 91.9 per cent., 90.8 per cent. It seemed, therefore, that this explanation was inadequate to explain the differential attack. Further, at the time when the work of Jones was carried out, comparatively little was known of the chemistry of the pectic substances, and the methods available for their examination were limited. For these reasons it was decided to make an investigation into the chemistry of the pectic constituents of the carrot, in an attempt to correlate this with the ease of attack by *B. carotovorus*, bearing in mind the considerations outlined below.

(1) Since rotting had been shown to be due to solution of the pectic substances of the cell-wall, and particularly of the middle lamella, differences in the chemical nature of the pectins of the two zones might exist.

(2) Some difference might be found in the nature of the nutritive substances present in the different tissues; e.g., the inner zone might contain more available nitrogen, leading to an increased proliferation of the invading bacteria, with consequent more rapid rotting of the host-tissue.

(3) The actual structure of the tissues might determine the ease of attack, the pectic substances being more or less protected mechanically by non-available tissues.

The problem obviously presents two aspects; a purely chemical aspect, involved in (1) and (2) above; while (3) involves a consideration of the structure of the root, in relation to the distribution of its chemical constituents. In the present work, only the purely chemical aspect has received consideration.

With this in view, the pectic substances of the two zones of the carrot have been prepared and examined by the methods usually adopted for determining the nature of such substances, i.e., by estimations of furfural yield, 'uronic anhydride' content, and methoxyl content. As the composition of the pectic substances is known to be affected by their mode of preparation, a standard procedure was adopted, in order that the samples should be truly comparable. The study of the protopectin presented a difficulty, in that no method has so far been devised for obtaining this substance in an unchanged condition free from cellulose. It was necessary therefore to convert this to soluble pectin by the usual methods, assuming

that any difference in the protopectin would be reflected in the soluble pectin prepared therefrom.

Determinations of the amounts of protopectin and middle lamella pectin present in the two zones were also made, since such data might indicate structural differences as suggested in (3) above.

Finally, samples of the pectin prepared from the two zones were submitted to the action of *B. carotovorus*, under controlled conditions, and the relative rates of decomposition were ascertained.

From the results obtained, it appeared that no difference between the pectic substances present in the cortex and the stele of the carrot could be detected by the ordinary methods of analysis. The furfural yield, uronic anhydride content, and methoxyl content did not vary beyond experimental limits, and were in agreement with the values accepted as normal for pectins prepared from roots (Table VI).

The distribution of the pectic and non-pectic substances in the two zones was found to be markedly different, the total pectin of the stele being approximately half that of the cortex. This difference was due to a difference in protopectin only, middle lamella pectin being practically the same in each case. The ratio of protopectin to middle lamella pectin seemed surprisingly high.

The experiments on the decomposition of the samples of pectin by means of *B. carotovorus* indicated that the organism was able to attack the pectic substances from each zone with equal ease; this supports the conclusion drawn from chemical analysis of the pectins, that they are identical in cortex and stele.

From the results obtained, it was concluded that any difference in the rate at which the two types of tissue of the carrot are attacked by *B. carotovorus* could not be explained on the grounds of a difference in the chemical nature of the substances attacked. Further examination of the manner in which the pectic substances are distributed throughout the tissues might be of interest in this connexion, while a study of the needs of *B. carotovorus* from the nutritional standpoint would be useful.

Experimental.

Preparation of pectin.

The carrots used were those obtainable commercially during the early spring. Such fairly old roots were found more convenient to use than young ones, since the differentiation between the two zones was much more marked—in fact, it was found possible easily to separate the two zones by splitting them apart, the partition (at the cambium) being quite clean and sharp. From about 5.5 kg. of fresh carrots, after cleaning and removing damaged portions, 3850 grm. of cortex and 1150 grm. of stele

were taken for working up. The method of treatment was the same in the case of each tissue. The tissue was rapidly minced and the juice pressed out; the press-cake was allowed to stand overnight with a large amount of cold water, in the presence of chloroform and toluene, and again pressed out. This process was repeated ten times, whereby all soluble matter was removed. After the final pressing the material was dried in air, pigments were removed by extraction with boiling alcohol, followed by a further washing with water, pressing, and drying. At this stage the weight of tissue from cortex was 210 grm. and from stele 78 grm.

The dry material was extracted in batches of 25 grm. with 750 c.c. portions of 0.5 per cent. ammonium oxalate solution at 85° for twenty-four hours. The pectin in the extract was precipitated in each case by the addition of three volumes of 95 per cent. alcohol. The precipitates were well washed with 75 per cent. alcohol, and purified by redissolving in water and reprecipitating as before. After further washing with alcohol, the products were dried first in air, powdered, and dried *in vacuo* over phosphorus pentoxide for twenty-one days. Care was taken that the tissues from the two zones were submitted to exactly the same treatment throughout. Yields of pectin obtained: cortex, 25.5 grm.; stele, 6.5 grm.

Portions of the pectin obtained from each zone were converted to pectic acid (or calcium pectate), since it was considered that this substance was obtainable as a definite entity, not showing the variations in composition met with in the soluble pectins. Calcium pectate was prepared by de-esterifying the pectin with dilute sodium hydroxide, acidifying with acetic acid, and precipitating with calcium chloride. In dealing with all products, the samples were dried to constant weight *in vacuo* over P_2O_5 ; ash estimations were made, and ash allowed for in the calculations of furfural, &c.

Furfural and uronic anhydride were determined on the samples of pectin and calcium pectate obtained from both types of tissue. Methoxyl estimations were carried out on samples of pectin prepared on a small scale under rigidly standard conditions, since it was known that the mode of preparation of a sample of pectin was liable to influence its methoxyl content. For this purpose, a fresh batch of carrot was used, 400 grm. of cortex and 200 grm. of stele being worked up. In each case the finely sliced tissue was boiled for twenty minutes with five times its weight of 95 per cent. alcohol, filtered off and dried, first *in vacuo* over sulphuric acid for twenty hours and finally at 90° in an oven for three hours. The dry material thus obtained was finely powdered in a coffee mill. 4 grm. samples of the dry material were extracted with boiling N/75 HCl for three hours; the extraction was repeated twice and the final residue washed with hot water. The pectin in the extract was precipitated by means of alcohol, and purified by redissolving in water and reprecipitating with alcohol. The product was finally dried *in vacuo* at 100° over P_2O_5 .

Throughout, the samples from cortex and stele were treated side by side in exactly the same manner. Extraction of the tissue with N/75 acid removes only the protopectin, leaving the middle lamella pectin unattacked. Since the middle lamella pectin is probably composed of completely de-esterified products, any variation in methoxyl content between the cortex and stele will be confined to the cell-wall pectin; it was for this reason that extraction with N/75 acid was adopted in preference to extraction with ammonium oxalate. For the determination of methoxyl, the micro method was used.

Table I summarizes the chemical constants of the pectin preparations obtained from the two types of tissue. The figures quoted are calculated on an ash-free basis. (The ash-content of the products varied from 0.5 per cent. to 2.0 per cent.) The ash-content of the samples of calcium pectate varied from 7.6 per cent. to 8.0 per cent.; the figures quoted for 'pectic acid' are in some cases based on analyses of calcium pectate, corrected for ash.

TABLE I.

		Cortex.	Stele.
Furfural yield	{ Pectin	19.10 %; 19.20 %	18.85 %; 19.06 %
	{ Pectic acid	20.84 %; 20.44 %	20.46 %
Uronic anhydride	{ Pectin	62.56 %; 62.64 %	63.32 %; 62.88 %
	{ Pectic acid	66.90 %; 67.13 %	68.17 %
Methoxyl	Pectin	9.61 %	10.93 %

Action of *B. carotovorus* :

The technique adopted was that described by Norman (11), who used a nutritive medium composed of 1 gram. peptone, 0.2 gram. potassium phosphate, and 0.1 gram. magnesium sulphate per 1,000 c.c. water; to this, pectin was added to give a concentration up to 1 per cent., and finally 2 gram. fine calcium carbonate was stirred in mechanically. Media were made up to contain the same concentrations of peptone, &c., and as nearly as possible the same concentrations of pectin, as indicated by an estimation of calcium pectate. Two sets of pectin media were prepared, one having an initial concentration of pectin (as Ca pectate) of 0.35 per cent., and the other, half this concentration; such media were made up with pectin from cortex and stele. 10 c.c. portions of the media were placed in tubes, sterilized by steaming for twenty minutes on three days, and inoculated with a loopful of culture of *B. carotovorus*, which had been subcultured on potato agar. The cultures were incubated at 30°. At the end of 2, 4, 7, &c., days tubes were removed, and the pectin remaining in the tubes estimated by the usual method. Control estimations were also carried out, with tubes of medium which were not inoculated. The results are summarized in Table II.

TABLE II.

Time of Incubation (days).	Wt. of Ca pectate from 10 c.c. medium.				
	Cortex I.	Stele I.	Cortex II.	Stele II.	Control.
	g.	g.	g.	g.	g.
0	0.0346	0.0324	0.0166	0.0164	0.0406
2	0.0235	0.0212	0.0108	0.0112	—
4	0.0148	0.0147	0.0083	0.0091	0.0389
7	0.0118.	0.0093	—	—	—
20	0.0094	0.0079	0.0062	0.0077	0.0416

It is evident from Table I that the usual methods of chemical analysis of pectins do not indicate any difference between the pectins of cortex and stele; while Table II shows that these substances, when isolated from the tissues, can serve equally well for the growth of *B. carotovorus* in culture media.

Distribution of pectic substances.

A number of experiments were performed in order to ascertain the distribution of the individual pectic substances in the various parts of the carrot. For these estimations, dried tissue was used, this being obtained in the manner described above. Soluble pectin, protopectin and total pectin were extracted and estimated by the methods suggested by Branfoot (1). Soluble pectin was extracted from fresh thinly sliced tissue by repeated extraction with cold water, after freezing the tissue for some hours; the amounts of calcium pectate obtained from these extracts were negligible. Protopectin was extracted by N/75 HCl, the dry tissue being boiled with the acid for three hours; each batch of tissue was extracted three times thus. Total pectin was estimated in the extract obtained by the action of 0.5 per cent. ammonium oxalate at 85° for twenty-four hours, followed by a thorough washing with hot water. 'Middle lamella' pectin figures were obtained by subtraction of 'protopectin' from 'total pectin'. The figures are summarized below:

TABLE III.

	% pectin (as Ca pectate); calculated on fresh weight.	
	Cortex.	Stele.
Soluble pectin	—	—
Protopectin	{ 2.817 2.813 } 2.815	{ 1.164 1.173 } 1.168
Total pectin	{ 2.852 2.959 2.919 } 2.910	{ 1.279 1.266 1.237 } 1.261
Middle lamella pectin	0.095	0.093

There is evidently a great difference in the amounts of pectic substances present in the two zones of the carrot, the outer zone containing rather more than twice the amount present in the stele. The difference is, how-

ever, confined to the amount of protopectin, i.e., pectin in combination in the cell-wall. The amount of middle lamella pectin is substantially identical in each case. Since the action of *B. carotovorus* is, initially at least, confined to the middle lamella, it does not seem possible to explain any differential attack of the two types of tissue on the basis of a difference of amount of middle lamella pectin available to the organism.

Note on nitrogen distribution in the tissues.

It has been pointed out above (p. 520) that some difference might exist in the nature of the dissolved nutritive substances present in the juice of the two zones. It has not been possible to examine this possibility at present, except to estimate, in one batch of carrot, the amount of total water soluble N in the cortex and stele. The results of a single experiment gave: total water soluble N in cortex—1.14 mg. per grm. fresh tissue; stele—1.11 mg. per grm. fresh tissue. Such a small difference cannot be regarded as of any significance.

SUMMARY.

1. Pectin and pectic acid have been prepared from the outer and inner zones (cortex and stele) of the carrot, and shown to be of the normal type in each case.

2. The distribution of the pectic substances in the two zones has been investigated; the cortex contains rather more than twice the amount of protopectin present in the stele; middle lamella pectin is the same in each case.

3. Differences in the rate of attack of the two types of tissue by *B. carotovorus* cannot be ascribed to any chemical difference between the pectic substances present.

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A Method for Measuring the Change of Permeability to Ions of Single Cells under Electric Stimulation.

(Preliminary Report)

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With one Figure in the Text.

WORKING with pulvinus of *Mimosa pudica*, Blackman and Paine (1) found in 1918 that after stimulation there was a slight, but definite, increase in the electrical conductivity of water surrounding the tissue. In a previous paper (4) I have shown that whenever a plant tissue is effectively stimulated, either mechanically or by an induction shock, its electric resistance diminishes, and this diminution of resistance is greater with increased intensity of the shock. It was there suggested that this diminution is correlated with an increased ionic permeability of the cells of the stimulated tissue. The micro-manipulation technique evolved by several workers (see Chambers (2)) makes it quite simple to test this conclusion directly by measuring, before and after stimulation, the electric resistance of a film of water immediately surrounding a single cell.

For these experiments *Nitella* is a very suitable material. Its several advantages are: (i) The large size of the cells, which, when isolated from the filament, retain their normal activity for a long time; (ii) the natural environment of the cells—fresh water makes it easy to detect changes in ionic concentration induced by minute quantities of electrolyte escaping from the cell; and (iii) the protoplasmic streaming movement of the cell, which affords an independent visible index of its physiological activity.

Experimental Arrangements.

A *Nitella* cell is placed on a mica slide between two electrodes; only a thin film of water is allowed to remain surrounding the cell, which is covered with liquid paraffin (nujol) to prevent any change of concentration due to evaporation or condensation. Platinum micro-electrodes, described elsewhere (5), were used to measure the resistance of the film of water before and after stimulation. These electrodes are simply micro-needles

of glass or quartz covered with a film of platinum. The best method for depositing such metal films is by spattering, but the simpler methods of Meillere's (3) for depositing bright platinum, and of Brashear's for silver also give reliable results.

Kohlrausch's method of measuring electrolytic resistance, in which alternating current has to be used, is not suitable for these experiments on account of leakage of current to the cell. The method used for measuring the resistance of the film of water was described earlier (4). When the micro-electrodes are brought into the film of water a deflection of the galvanometer is observed, due to the contact e.m.f. (a few millivolts) from the leads, the electrodes, and the film of water surrounding the cell. For each observation the total e.m.f. of the circuit, however induced, is balanced with an equal and opposite e.m.f. from a potentiometer. The current sensitivity of the Leeds and Northrup galvanometer used in these experiments was 30 mm. per 10^{-9} ampere. From the deflection of the galvanometer and the readings of the potentiometer the resistance of the circuit was calculated. The resistance of the electrodes, the leads, and the galvanometer was negligible.

A trough was built on a thin sheet of mica of the size of about 15×5 mm. and 0.5 mm. deep. Such a trough is easily built when an exact geometrical shape is not essential by holding the mica slip over a low flame and placing on it fine threads of De Kotinsky cement. The electrodes for stimulation consists of fine platinum wires fixed on to, and running parallel with, the ends of the trough, and are led out through holes in the mica to the other surface and soldered to an equally fine copper wire, to prevent transmission of mechanical vibration to the film.

A *Nitella* filament is first cleaned with a fine camel's-hair-brush, and young cells of about 10 to 12 mm. in length are isolated under water and then transferred to a large dish of clean spring water, where they are kept for an hour to allow the soluble contents of the cut cells to diffuse out. After that the cell is placed with its ends on the stimulating electrodes (Fig. 1). The excess of water is drained off by a piece of filter paper, and the preparation is then covered with paraffin and inverted with a rapidly sharp twist and mounted over a moist chamber. A *Nitella* cell in a hanging drop like this will show normal streaming movement for several hours.

For operating the micro-electrodes Chambers's micro-manipulators were used. The electrode tips are brought up into the film of water and are generally placed about 100μ apart, and each about 10μ from the cell-wall. For the purpose of these experiments, where no attempt is made to measure the specific resistance of the film of water, the essential thing to be secured is that the resistance of the lead-off circuit should not vary during the course of observation. The leads from the film of water being all metallic in the above arrangement this condition is fulfilled. The cells

are stimulated by an induction shock, whose duration is controlled by a metronome and intensity varied by the position of the secondary coil. The galvanometer being highly sensitive a very high insulation of the circuit is essential.

Experimental Results.

The resistance of the film is measured after the cell recovers from the handling of mounting in the hanging drop, i.e. after the velocity of the

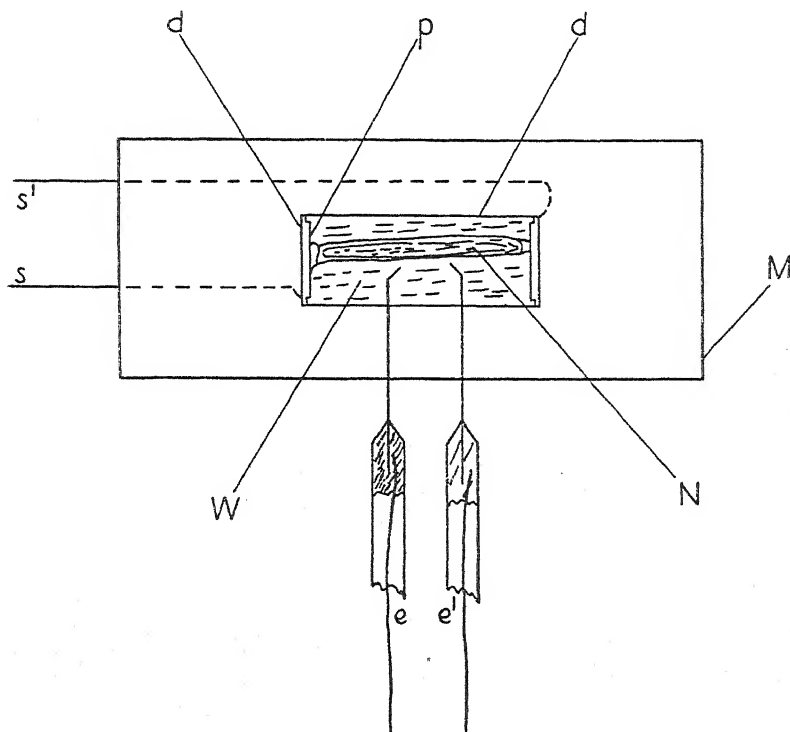


FIG. 1. Diagram of experimental arrangement as seen from above. *m*, mica cover-slip; *N*, *Nitella* cell; *d*, De Kotinsky cement wall; *p*, platinum wire for stimulation; *s, s'*, fine copper wire leads; *w*, water film; *e, e'*, micro-electrodes.

streaming movement attains a steady value, which is measured by observing the time it takes for a granule to travel 100 divisions of the micrometer eyepiece of the microscope, and its rate in μ thus determined. The resistance of the film of water remains fairly steady for nearly two hours. When the cell is stimulated by a sub-minimal shock, i.e. when the streaming movement is not stopped by a shock, hardly any change of resistance of the film of water could be observed. Only when the intensity of the shock induced a stoppage of the streaming movement did a diminution of resistance of the film of water follow. A cell could be stimulated 5 to 6 times with a minimal stimulus, i.e. one which just induced cessation of

streaming. After each stimulation there would be a diminution of resistance of the film of water concomitant with the stoppage of streaming movement. For such minimal stimuli a decrease of about 20 per cent. in the resistance of the film of water was observed. When the intensity of the shock was increased, the observed diminution of resistance was greater. At a lethal shock a diminution of resistance of about 300 per cent. could be observed. Over ten different specimens were experimented upon, and in every one of them similar phenomena were observed. The detailed observations on one typical specimen are given below.

Experiment 1.—The size of the *Nitella* cell was 11 mm. long and its diameter 0.296 mm. The velocity of normal streaming was 34 μ per sec. The tips of the electrodes were 5 and 6 μ respectively in diameter, and were placed each 10 μ from the surface of the cell, and were 108 μ distant from each other. The combined resistance of the micro-electrodes used was 102 ohms. The results are shown in Table I.

TABLE I.

Change of Electric Resistance of Water Film surrounding Nitella Cell before and after Stimulation.

(I) Intensity of induction shock given in coil distance; (V) velocity of streaming; (μ per sec.); (R) resistance of water film; (D) the observed diminution of resistance.

Time.	Condition of Specimen.	(I).	(V).	Resistance (10,000 ohms.)	
				(R).	(D).
3.20 p.m.	Normal		34	426	
3.21 "	Stimulated	11 cm.	0	382	84
3.26 "	Recovered		32	401	
3.27 "	Stimulated	11 "	0	322	79
3.32 "	Recovered		31	389	
3.33 "	Stimulated	5 "	0	294	95
3.40 "	Recovered		26	324	
3.41 "	Stimulated	1 "	0	106	218
4.10 "	No recovery		0	118	
4.15 "	Stimulated	1 "	0	109	9

From the table it will be seen that whenever the cell is so stimulated as to stop the streaming movement there is a decrease of resistance of the water film, which increases with greater intensity of the shock. Further, it will be seen that when the coil distance was only 1 cm. the shock killed the plant, and the killed specimen subjected to another shock of lethal strength showed only a slight decrease of resistance due to the heating effect of the induction current.

Experiment 2.—To find out the extent of change of resistance brought about by purely physical effect of the stimulus, and from other sources, control experiments were carried out with a *Nitella* cell, previously killed

by puncture or chloroform (washed thoroughly in spring water before mounting on the stimulating trough), and subjected to lethal shocks. The resistance of the film was of the order of 4 megohms and the diminution of resistance observed averaged only 5 per cent.

Conclusions.

The experiments described above, though they have no quantitative significance, show that whenever a *Nitella* cell is effectively stimulated the ionic concentration of the medium surrounding the cell increases, which under the conditions of the experiment can only be ascribed to exosmosis of ions through the protoplasmic membrane; in other words, the permeability of the protoplasmic membrane to ions increases whenever a cell is effectively stimulated. Experiments are being devised for securing quantitative results and also the effects of different ions on permeability of the protoplasmic membrane; this will form the subject of future communication.

Summary.

Experiments are described in which the electric resistance (to direct current) of a film of water surrounding a *Nitella* cell is measured with micro-electrodes, before and after stimulating the cell with induction shocks of varying intensities.

These observations show that whenever the *Nitella* cell is effectively stimulated the ionic permeability of the protoplasmic membrane of the cell increases.

I take this opportunity to acknowledge the generous financial help received from Mr. and Mrs. L. K. Elmhirst in the form of a travelling scholarship. I am indebted to Prof. Robert Chambers and his staff for their unfailing kindness and the facilities extended to me at the Washington Square College, New York, for acquiring the technique of micro-dissection and for carrying out this investigation, and to Prof. V. H. Blackman for help with the manuscript.

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NOTES.

NOTE ON THE LIFE-HISTORY OF CLADOPHORA FLAVESCENS, KÜTZ.—In a recently published paper entitled 'Reduction Division in a species of *Cladophora*' (this Journal, clxxv, July, 1930, pp. 587-92), the writer described reduction division occurring in the sporangial initials of a *Cladophora* species, stated to be probably *C. flavescens*. In March, 1930, *C. flavescens* was collected from some brackish pools on the sea-shore at Pooyl Vaaishe on the south-west coast of the Isle of Man, where it was growing in abundance. Ripe sporangia were of common occurrence on this material, and spore release was readily obtained. Although most of the released zoids, after swimming for about twenty minutes or less, settled to the bottom of the culture dish without fusing, some fusions were observed. These fusions usually took place between pairs of zoids, but occasionally three or more zoids became attached to one another and frequently fused end to end to give a curiously shaped body which exhibited peculiar rolling and lurching movements.

Fixings of this material were made throughout a day and night, a chrom-acetic mixture being used. Preparations of the unsectioned material were made and stained with Heidenhain's iron haematoxylin. Examination showed that nuclear division had taken place in the evening, nuclear figures being found most frequently in material fixed during the interval between dusk and midnight, and were particularly numerous between the hours of 8.0 p.m. and 10.0 p.m.

The cytological, as well as the morphological details of the material show that the previously described species, reference to which has been made above, is identical with *Cladophora flavescens*, Kütz. It is therefore unnecessary to give any further description of the cytological details here. The cytological examination has shown, however, that here, as in the species described by Schussnig (Berichte der Deutschen Botanischen Gesellschaft, xlv, pp. 481-90, 1928 and xlvii, pp. 266-74, 1929), there occur two morphologically similar but cytologically distinct generations, the vegetative nuclei of the diploid plants having twenty-four chromosomes and those of the haploid plants, twelve chromosomes. The two generations appear to have grown very closely mingled together, since any one fixing has usually proved to contain specimens of both haploid and diploid plants.

So far, an examination of the fixed material has revealed no case of a haploid plant bearing reproductive organs. The diploid plants, on the other hand, usually bear sporangia in whose initials all the phenomena of reduction division are seen.

The behaviour of spores on release requires some further elucidation. The rarity of fusions between the released zoids is in accordance with the observed fact that all the sporangia investigated cytologically have proved to be borne on diploid plants and therefore would be expected, since the plant has two generations, to give

rise to asexual zoospores. The presence of a certain number of fusions, however, involves the acceptance of one of two alternatives. Either some haploid material bearing ripe gametangia must have been present amongst the specimens from which spore release was obtained, or else the haploid zoospores from the diploid plants must have some sexual potentialities, and may under certain conditions act as gametes and fuse. It appears to the writer that the second alternative is the most feasible for the following reasons:

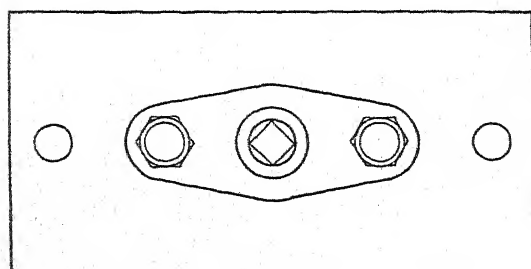
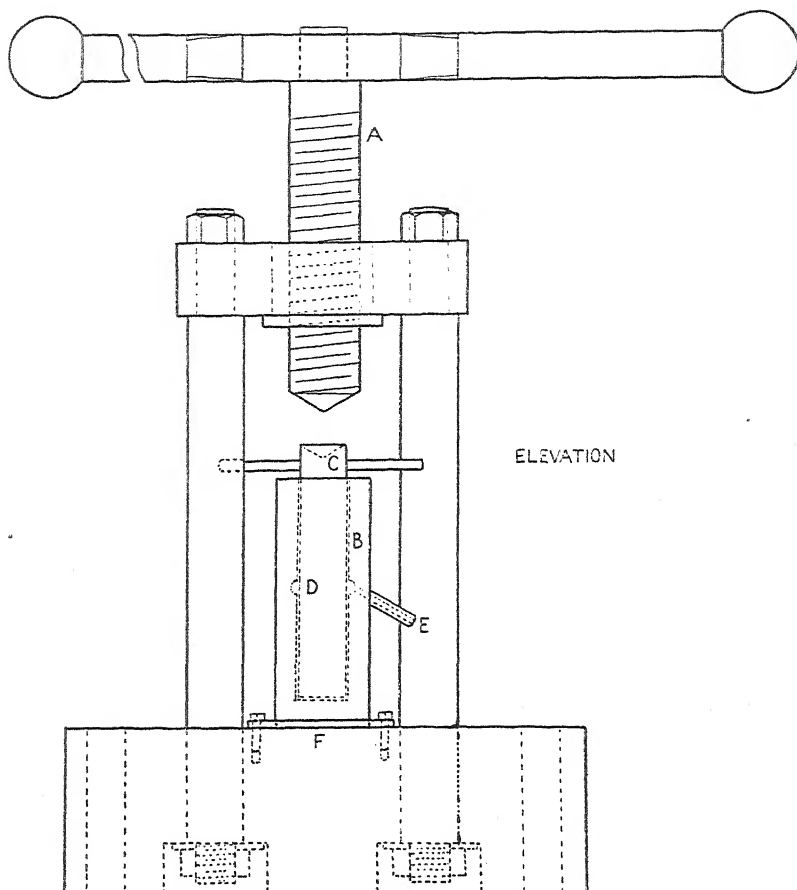
1. The absence of fertile haploid plants amongst any of the fixed material examined makes it appear improbable that they were actually present in sufficient numbers to account for the number of fusions seen.

2. Since, for purposes of observation, spore release was usually obtained from a small fragment of material on a microscope slide, it is unlikely that fragments of more than one plant would be present in nearly every case in which fusions were seen, since at least one was seen in the majority of cases.

3. In many cases sporangia which had released apparently asexual zoospores, retained some zoids which had apparently been unable to make their escape, and, between these, fusions were often observed within the sporangium wall, indicating that the haploid zoospores have a gametic potentiality which is capable of becoming apparent under certain conditions.

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0 1 2 3 4 inches
Scale

NOTE ON A CONVENIENT PRESS FOR SAP EXTRACTION.—For certain branches of physiological and biochemical research an efficient press of small capacity for the expression of sap from small quantities of vegetable tissue is a necessary piece of apparatus.

Presses for this purpose, though frequently forming part of laboratory equipment, are not always as efficient in operation, or as convenient to manipulate as one would desire.

Some years ago the writer had occasion to design a press for use in connexion with some work on osmotic pressure of the cell-sap of leaves. Preliminary sketches were prepared, and with the aid of Mr. R. C. Porter, Lecturer in Engineering at the University of Birmingham, the apparatus described below, which worked admirably, was evolved.

It will be seen from the figure that the main part of the machine consists of an ordinary screw press of the orthodox type which is capable of exerting a pressure of several tons.

The screw *a* operates on the sap-extracting apparatus which consists of a cylindrical barrel *b* fitted with a plunger *c*, both of which are machined from stainless steel.

The bore of the barrel was made 0.0015 inches larger than the diameter of the plunger. This is sufficient to allow the latter to slide freely into the former, giving an annular clearance space between the two of 0.00075 inches. About half way up the bore of the barrel an annular groove *d*, one-eighth of an inch wide, is cut which communicates with the outside by means of a small side tube *e*. The upper end of the plunger is fitted with a cross-bar which serves for a handle, and the top has a conical recess into which fits the point of the screw. When in position in the press, the barrel is orientated centrally by means of a metal plate *f* bored out to fit its base, and screwed to the base of the press.

When using the apparatus for sap extraction, the tissue to be operated upon is tied up in a small piece of muslin or similar fabric and placed in the barrel. The plunger is introduced into the barrel and the whole fitted in place in the press. Application of pressure by means of the screw causes the sap to be forced up past the plunger as far as the groove in the barrel from which it is run off through the side-tube into a beaker or similar receptacle.

Although the apparatus here described is of comparatively small capacity, there is no reason why the main dimensions should not be varied to meet any special requirements.

The important features of this press are : (*a*) very little sap is lost owing to the small clearance between the barrel and the plunger, and (*b*) the expressed sap is run off while the pressure is being applied, and has not to be subsequently poured out of the apparatus.

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W. LEACH.

On the Morphology and Ecology of *Ranunculus parviflorus*, L.

BY

E. J. SALISBURY.

With Plate XVIII and twenty Figures in the Text.

THE discovery that the Ranunculaceae exhibited a hidden trimery (cf. E. J. Salisbury (16, 17)) was primarily responsible for directing the writer's attention to this species. In contrast with the types already investigated, *Ranunculus parviflorus* has a flower consisting of a small total number of parts whilst the corolla consists of few petals, or may be entirely wanting. The sequel showed that *R. parviflorus* is interesting from so many standpoints that every opportunity was taken to study the plant in its native habitats and in cultivation. The result has been to obtain a fairly complete picture of the life-history and biology of the species, and has brought to light a number of interesting facts which appear to be of sufficient importance to warrant their detailed description.

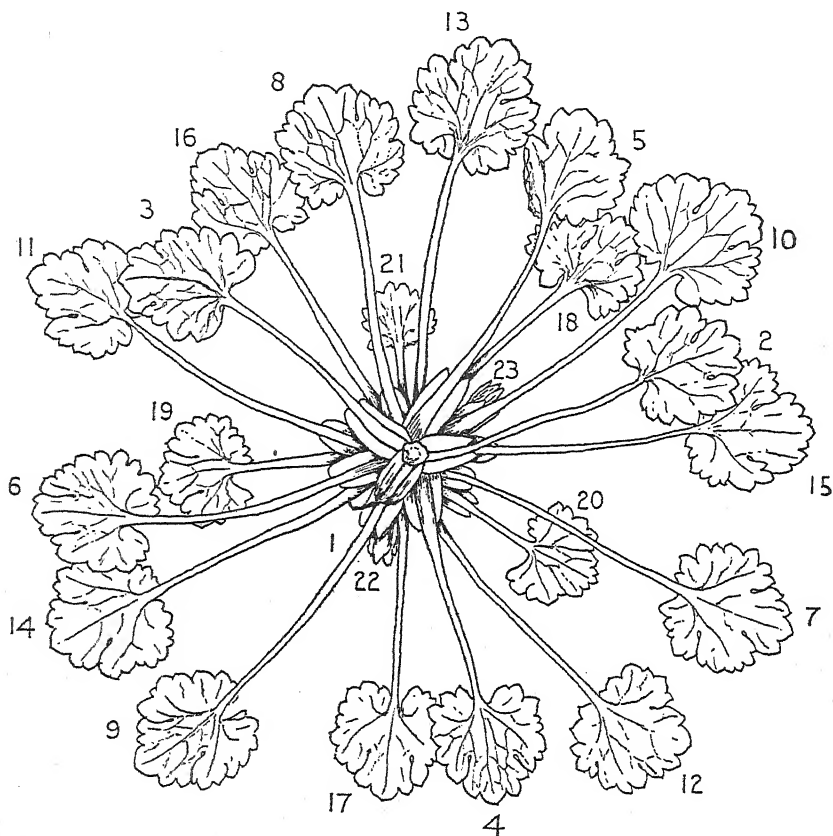
The material on which this study is based has partly been obtained from wild habitats, but in addition the species has been cultivated under various conditions for a period of ten years, which has enabled the life-history to be ascertained with considerable accuracy.

Most of the wild material was obtained by the writer from Cornwall, but I am indebted to Dr. Eric Drabble for specimens from the Isle of Wight, to the Rev. W. Martin for plants from Devonshire, and to Dr. W. Watson for material from Somerset. I am also indebted to the Linnean Society, the Botanical Department of the Natural History Museum, and the Kew Herbarium for facilities for the examination of herbarium material.

Morphology and Anatomy.

(i) *Vegetative.* The leaves of *R. parviflorus* form a well-defined rosette on what is usually a short axis. If such a rosette is carefully examined, it will be found that the phyllotaxy is far from regular and varies greatly as we pass from the first formed leaves to the most recently

produced. In Text-fig. 1 a typical example of a winter rosette is portrayed as seen from below. The lower leaf (1) has withered, and only the leaf-base remains, but the angle of insertion of the successive leaves,



TEXT-FIG. 1. Winter rosette of *Ranunculus parviflorus* as seen from below. The leaves are numbered in sequence from below upwards.

numbered in the order of their position, can be clearly determined from leaf 1 to leaf 23. The angle between each successive pair of leaves is shown in Table I, opposite.

It is at once apparent that, though the average, viz. 137.3° approximates to a $3/8$ phyllotaxis (135°), and the ninth leaf as seen in Text-fig. 1 is approximately superimposed above the first, yet this gives a false impression of regularity, since the angular divergences in reality exhibit a wide range of from 121° to 154° .

The conventional expressions of $2/5$ and $3/8$ phyllotaxy should mean a regular interval of 144° or 135° , but actually this is not the case, and hence they merely express the fact that two turns of the stem in the one

case involve six leaves and that in the other three turns involve nine leaves ; the more crowded the leaves the greater the irregularity, and hence the more intervals necessary to obtain an average approximating the true mean.

TABLE I.

Angular Divergence between Leaves.

Order of leaf from below upwards.	Angular divergence.	Order of leaf from below upwards.	Angular divergence.
1-2	132°	11-12	129°
2-3	130°	12-13	154°
3-4	140°	13-14	122°
4-5	144°	14-15	150°
5-6	129°	15-16	121°
6-7	140°	16-17	139°
7-8	130°	17-18	148°
8-9	123°	18-19	132°
9-10	153°	19-20	136°
10-11	131°	20-21	135°
		21-22	153°
		22-23	151°

Range, 121° to 154°. Average, 137.3°.

A more accurate estimate of the mode of insertion of the leaves is obtained from transverse sections of buds. For this purpose use was made of serial sections, and the angular divergence was measured with reference to the centre of the main axis and the median petiolar bundle of each leaf. Measuring the divergences in this way of the leaves in Text-fig. 3, the following values were obtained :

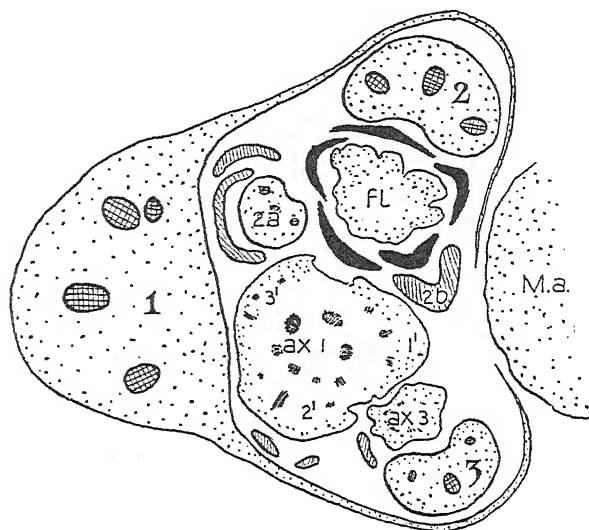
Order of leaves.	Divergence.
1-2	139°
2-3	140°
3-4	130°
4-5	141°
5-6	130°
6-7	148°
7-8	123°
8-9	126°
9-10	159°

Range, 123° to 159°. Average, 137.3°.

In another bud (Text-fig. 2), the intervals were a very close approximation to 120°. The fact that the averages in the two first instances cited, though based on different methods, are identical to the first place of decimals is, of course, a coincidence, but this merely emphasizes that the degree of dispersion is similar on different plants.

The important fact that emerges is that the angular divergence is a very variable character, and that to state that the phyllotaxis is $\frac{3}{8}$ or $\frac{5}{13}$ is merely a convenient fiction, and, like many averages in biological

phenomena, is not represented in any of the actual records. This is more clearly realized from the diagram in Text-fig. 4, where the angular divergences between fifty-five pairs of leaves are plotted as a series of vertical lines, proportional in height to their divergences, arranged in order of



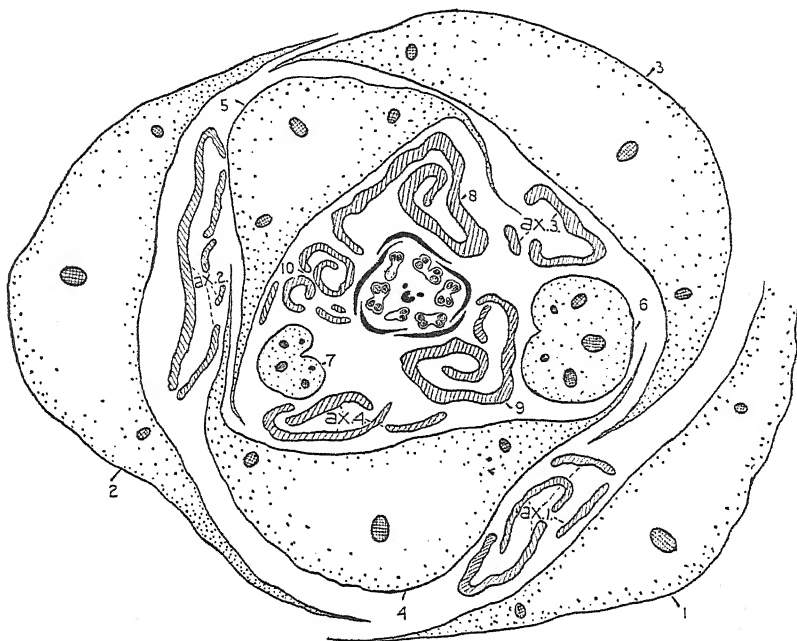
TEXT-FIG. 2. Transverse section of a young shoot showing the arrangement of leaves at an angle of approximately 120° . *M.a.*, Major axis. The axillary shoot bears the leaves 1, 2, and 3, and the leaves represented by the leaf-traces 1', 2', and 3'. The bud axillary to leaf 2 bears the leaves 2a and 2b, and the terminal flower *fl*.

magnitude. The horizontal lines represent the angular divergences of the more frequent types of phyllotaxy. The actual records range from 89° to 159° . There are three examples corresponding to a divergence of $1/3$, and one example each of divergences corresponding to a $3/8$, $2/5$, and $8/21$ phyllotaxy. The majority, amounting to nearly 54 per cent., lie between $1/3$ and $3/8$, whilst 9.2 per cent. exhibit divergences under 105° .

It is evident that, with such marked variation, no importance can be attached to the occurrence of any particular angle, even though there would appear to be something approaching a mode in the region of 120° . Indeed, the only common factor, in view of the wide range, is one that is shared by all the common divergences exhibited by Dicotyledons, namely, the fact that, *in any one complete turn of the spiral sequence around the stem, there are always three leaves present.* Lateral displacement, due to mechanical or other causes, brings about the variety of divergences present in the species, or even in the same individual, but whatever the result of such displacements may be, and usually this tends towards an increase in angle, three lateral members invariably occur in each turn, and this is the true significance of the Fibonacci series.

Some time ago the author suggested that the apical meristem of the

angiosperm might perhaps be a multicellular derivative of a three-sided apical cell, which, as a consequence of its phylogeny, normally tends to produce lateral members to the number of three in every turn (18). It is at once apparent that such an interpretation would be consistent with the

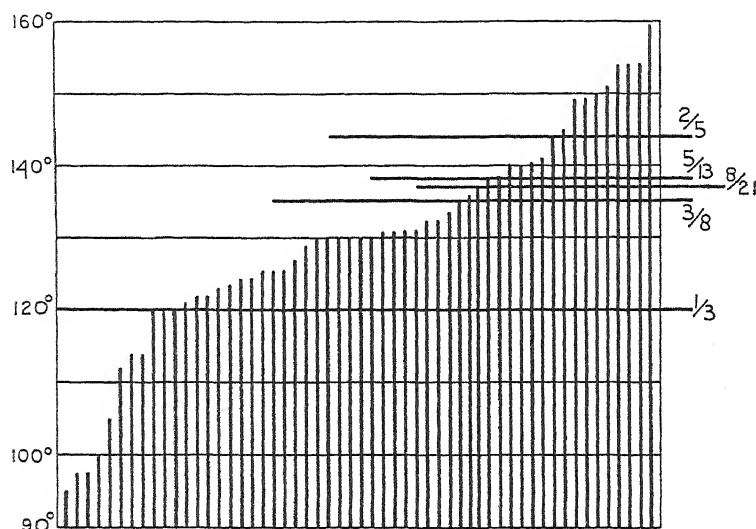


TEXT-FIG. 3. Transverse section through the rosette of *R. parviflorus* showing the leaf arrangement and the terminal flower in which there are present six stamens in two whorls of three each, and the three uppermost carpels are also cut through at this level. Leaves numbered in sequence from below upwards. ax. 1, ax. 2, ax. 3, leaves of axillary buds.

facts just adduced and would explain the common factor in the diversity of leaf arrangements encountered. Moreover, in rapidly growing specimens of *R. parviflorus*, in which the elongation of the axis takes place with sufficient rapidity to reduce the lateral displacement of leaf rudiments through mechanical pressure, a divergence of $1/3$ (120°) may be the rule. An example is shown in Text-fig. 2. In this specimen the angles between leaves 1 and 2, and 2 and 3, are 120° , and the same $1/3$ phyllotaxy is shown by leaves 2 2a, and 2b, as also by the petiolar traces in the secondary axis (ax. 1). From an examination of sections through several specimens it is evident that departures from a $1/3$ arrangement are explicable on the basis of the space relations of the rudiments of the lateral organs at the growing point and the consequent mechanical displacements.

The leaf. The leaf is typically trilobed and more or less reniform in general outline, with a well-marked differentiation into leaf-base, petiole, and lamina. It is not infrequently stated that the Ranunculaceae do not

exhibit stipules, and though this is manifestly untrue for the genus *Thalictrum* and the *Batrachian Ranunculi*, yet most of the yellow-flowered terrestrial buttercups do not develop stipules. In *R. parviflorus*, however, the leaf-base has a broad membranous wing on either side, and these wings



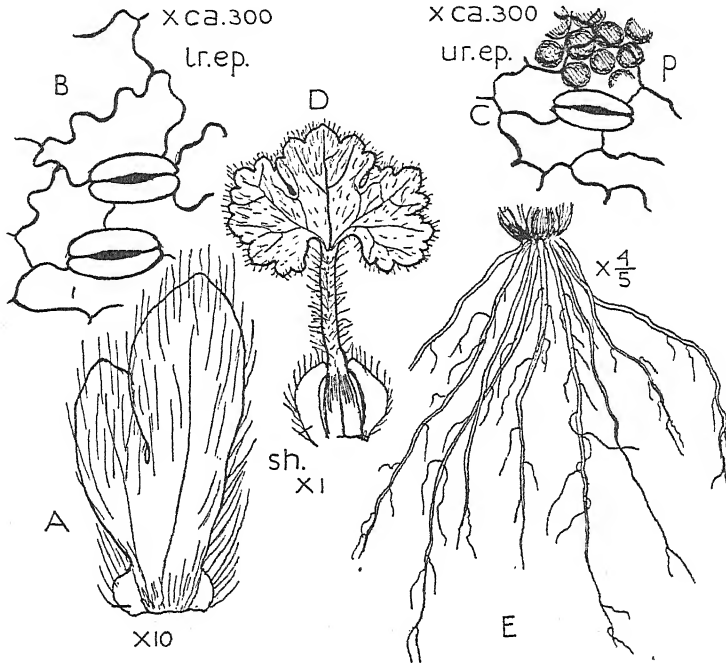
TEXT-FIG. 4. Angle of divergence between fifty-five pairs of leaves arranged in order of magnitude, showing the wide range of variation with the mode in the region of 120° .

become free at their apices, so that the leaf-base may be said to possess adnate stipules (cf. Text-fig. 5, D). The leaf-base receives three vascular strands, viz. a median and two lateral strands. These latter both branch, so that in the petiole the number of strands is five. The ground tissue is noteworthy for the numerous and conspicuous intercellular spaces, comparable to the lacunar system of a marsh plant (Pl. XVIII, Fig. 7).

The leaf margin is crenated and the tip of each crenation is occupied by a pale patch of rather yellowish opaque tissue, which marks the position of the epithem beneath a group of, usually 1–3, water-pores. These are very pronounced in the young leaf, and are already differentiated in the quite juvenile leaf within the unopened bud. The very young leaf is also densely clothed with silky hairs, which become separated as the leaf expands, but persist to maturity. Similar hairs also occur on the petiole and the margin of the membranous stipules. The young leaf is thus well protected against the action of drying winds, but for this very reason the rate of transpiration is reduced so that in wet weather there is liability of excess of water, for which the extensive system of water-pores offers a safety valve.

An epinastic curvature of the petioles results in the laminae being pressed close against the soil, and like most rosette leaves they bear

stomata on both surfaces. The stomata are more numerous below, where they number usually between 50 and 90 per sq. mm. in sun plants. On the upper surface they number from 14–18 per sq. mm. The cauline leaves, as is to be expected (cf. Salisbury (20)), present higher numbers, usually

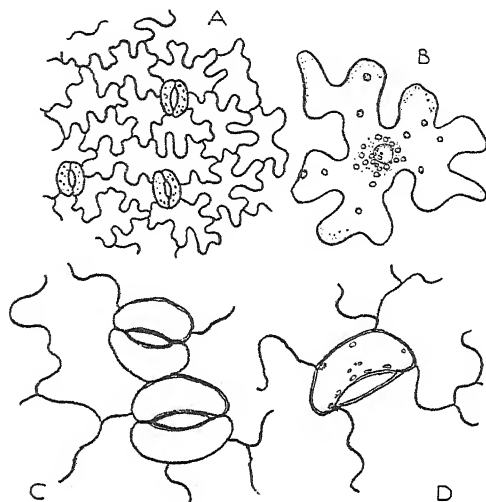


TEXT-FIG. 5. Details of vegetative structure. A. Herbaceous sepal for comparison with D. B. Lower epidermis. C. Upper epidermis. D. Leaf from rosette. E. Root system. *p.*, palisade cells. *sh.*, leaf sheath showing stipular appendages.

from 12–20 per sq. mm. in the upper epidermis and about 88 per sq. mm. in the lower. Both upper and lower epidermal cells exhibit undulating vertical walls, a feature which is slight above but marked below, even in sun-leaves (cf. Text-fig. 5, B and C), and which is least pronounced in plants growing in full sunlight and very dry situations. The sinuosity is most marked in shade-leaves and on plants growing in moist conditions, where the epidermal cells assume so irregular a form as to present the appearance of a complicated jig-saw puzzle (Text-fig. 6, A and B).

The epidermis of a very young leaf in the unexpanded bud bore 4,140 stomata per sq. mm. and 40,680 epidermal cells per sq. mm., with a stomatal index of 9.2. At this early stage, when the individual cells are only about 0.000022 sq. mm. in area, or less than $1/210$ of the area which they attain in the adult leaf, the stomatal mother-cells already show differentiation into two guard-cells, and in some, though by no means all, the pore is already formed. The epidermal cells at this stage are mostly

polygonal, as seen in surface view, with almost vertical walls (Pl. XVIII, Fig. 11). But even in this immature condition there are very slight indications of the inception of the sinuosities which become so extreme in these vertical walls in the adult lamina. The slight sinuosity that may be



TEXT-FIG. 6. Details of epidermal structure. A. Lower epidermis showing sinuosity of the vertical walls. B. A single cell. C. Twin stomata. D. A half stomata. A x about 100, B ca. 200, C and D ca. 300.

exhibited by adult epidermal cells of sun-leaves is thus a retention of a juvenile character, and this is true also, as the writer has elsewhere shown (20), of the higher stomatal frequency which characterizes sun-leaves.

The irregularity of the stomatal orientation, which is so marked a feature of most, though by no means all, Dicotyledonous leaves, might be thought to be an outcome or at least correlated with the irregular form of the adult epidermal cells, especially since the regular orientation of the stomata, which characterizes most Monocotyledonous leaves, and a few Dicotyledonous ones (e.g. *R. gramineus*; *Silene maritima*), is commonly associated with epidermal cells which are regular in outline. That this is not so is indicated by the fact that, in the very immature epidermis of *R. parviflorus*, whilst the cells are still almost regular and even exhibit a distinct serial tendency, the stomata are not regularly orientated, and although the stomata in general tend to be formed with the plane of division between the guard-cells approximately parallel to the midrib of the leaf, or leaf lobe, yet an appreciable proportion have their axes inclined at an angle which may be as large as 45° from the axial direction. Actually, the orientation of the stomata in the adult lamina of this species is often much more regular than is usual in Dicotyledonous leaves, and the data in the accompanying table show that it is doubtful whether, despite the extremely irregular form

of the adult epidermal cells of this species, the orientation of the stomata in the mature epidermis is significantly more irregular than it is at the time of stomatal differentiation when the epidermal cells are still almost polyhedral (cf. Table III, p. 548).

TABLE II.

Distribution of Stomata and Stomatal Frequency. Ranunculus parviflorus Seedlings grown in Shade.

	Cotyledons.	1st leaf.	2nd leaf.	Immature leaf.
<i>Plant A.</i>				
Stomatal frequency . . .	18-30	33-36	—	—
Epidermal cells per sq. mm. .	133-165	210-213	—	—
Stomatal index average . . .	11.5	14.0	—	—
<i>Plant B.</i>				
Stomatal frequency . . .	10-13	28-40	—	—
Epidermal cells per sq. mm. .	142-158	200-224	—	—
Stomatal index average . . .	7.1	15.8	—	—
<i>Plant C.</i>				
Stomatal frequency . . .	4-12	58-93	—	—
Epidermal cells per sq. mm. .	149-180	300-342	—	—
Stomatal index average . . .	5.3	18.7	—	—
<i>Plant D.</i>				
Stomatal frequency . . .	1-12	13-39	27-53	—
Epidermal cells per sq. mm. .	89-137	214-268	194-242	—
Stomatal index average . . .	5.77	9.4	15.5	—
<i>Plant E.</i>				
Stomatal frequency . . .	—	17-28	39-43	—
Epidermal cells per sq. mm. .	—	210-236	206-252	—
Stomatal index average . . .	—	9.0	15.2	—
<i>Plant F.</i>				
Stomatal frequency . . .	9-15	27-28	5-23	—
Epidermal cells per sq. mm. .	75-86	170-205	160-178	—
Stomatal index average . . .	12.6	13.2	8.0	—
<i>Plant G.</i>				
Stomatal frequency . . .	5-11	53-54	50-52	153
Epidermal cells per sq. mm. .	99-125	163-198	127-173	760
Stomatal index average . . .	7.5	22.2	25.7	16.7

Sun Plants.

Stomatal frequency, 68-90. Epidermal cells per sq. mm., 206-397.
Stomatal index average, 16.5 (15.4-24.8).

TABLE III.

Showing Percentage of Stomata with Irregular Orientation in Very Immature and Adult Leaves.

	% with approximately parallel orientation.	% Irregular.
Immature leaf A	67	33
" " B	72	28
" " C	83	17
Mature leaf A	94	6
" " B	67	33
" " C	80	20
" " D	46	54
" " E	67	33
" " F	48	52
" " G	69	31
Immature leaf, average:	74	26
Mature leaf, average:	67.3	32.7

As seen in transverse section (Text-fig. 7), the epidermal cells appear to consist of two types, namely, very large cells and relatively small ones, the latter occurring two or more together. These apparently small cells are, however, in actual fact, the lobes of the large cells cut across, and since the lobes of adjacent cells interdigitate, so that in vertical sections of the leaf two or three such sectioned lobes usually appear together, the larger vertical sections where the main body of a cell has been cut through thus alternate with the apparently small cells.

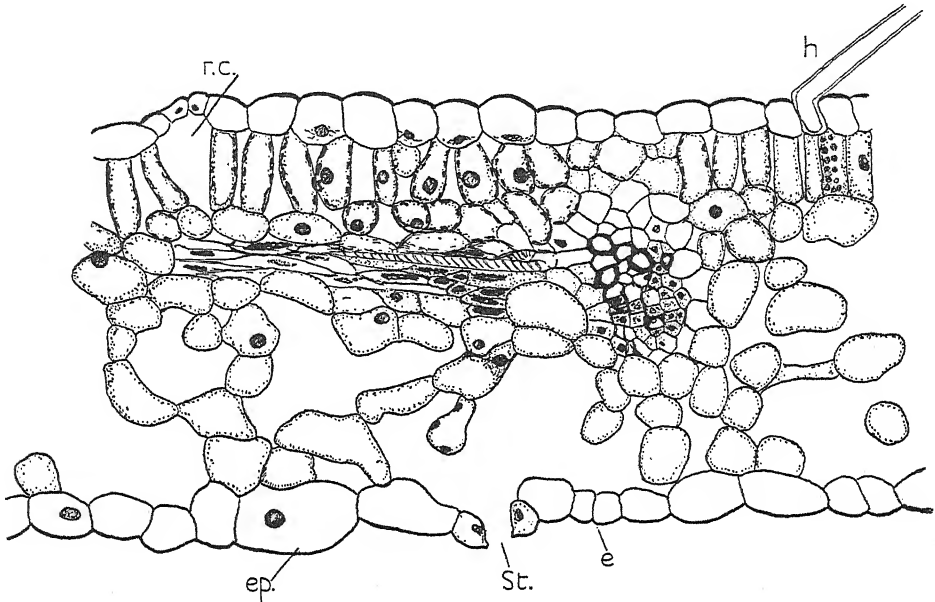
A study of the stomatal frequencies of seedling plants from successive leaves (Table II) shows these to range from 1 to 30 in the cotyledons (average, 11.7), in the first leaves from 13 to 93 (average, 39), and in the third leaf from 5 to 53 (average, 35). In seedlings grown in full sun, the frequency ranged from 68 to 90. The stomatal indices are seen to range from 5.3 to 12.6 in the cotyledons (average, 8.3), from 9.0 to 22.2 in the first leaves (average, 14.6), and in the second leaves from 8 to 25.7 (average, 16.1). The *proportion* of stomata to epidermal cells thus would appear to increase in the seedling from below upwards. The average stomatal index for the second foliage leaf of seedlings grown in the shade is, it will be noted, not significantly different from the average index (viz. 16.5) for 'sun-leaves' of adult plants.

The observed range of stomatal index is comparable with those of plants of the woodland flora, and does not suggest a plant adapted to arid conditions, a feature of some interest in view of the atlantic distribution of the species (cf. below, p. 570).

There is a single palisade layer constituting about a quarter of the total thickness of the chlorenchyma, interrupted here and there by a large

respiratory cavity situated beneath a stoma in the upper epidermis. The spongy tissue of the radical leaves is very lacunar, as also is the cortical tissue of the petiole (Pl. XVIII, Fig. 7).

The average size of the stomata when fully turgid is $63\ \mu \times 52\ \mu$ and of the actual pore $34\ \mu \times 18\ \mu$. If we take the normal stomatal frequency as



TEXT-FIG. 7. Transverse section through a rosette leaf showing the very lacunar structure the raised stomata (*st.*) and the apparently very unequal epidermal cells. The main body of the cell has been cut through at *ep.* whilst at *e* the arms of the sinuous cells have been sectioned (compare Text-fig. 6, A and B). *h.*, hair, *r.c.*, respiratory cavity beneath a stoma in the upper epidermis. \times ca. 200.

ranging from about 30 to 80 per sq. mm. according to the aridity of the environment, then the aggregate area of stomatal apertures per sq. mm. of leaf surface ranges from about 0.0126 sq. mm. for shade-leaves to 0.036 sq. mm. for sun-leaves; or, roughly, between 1 per cent. and 3 per cent. of the total leaf surface. Abnormal stomata with only one guard-cell in which the aperture is plano-convex (cf. Text-fig. 6, D) and twin stomata (Text-fig. 6, C) have been met with.

The stomata in transverse section are seen to project above the general level of the epidermis, and in this respect and in the form of the guard-cells resemble those of a marsh plant rather than of a xerophyte. It must be emphasized in this connexion that the radical leaves are pressed close to the ground, except in plants grown in shade, and chiefly function during the wettest season of the year. Nevertheless, it is noteworthy that the lacunar tissue of petiole and lamina, the stomatal position and structure, and

the great irregularity of the epidermal cells even in 'sun-leaves', all suggest a plant of damp, rather than dry, habitats.

The root system is a shallow fibrous one, attaining a depth of about nine to ten centimetres, and in transverse section the roots show a tetrarch (diarch in the seedling) vascular strand surrounded by a cortex, in which the numerous and conspicuous lacunae of the middle region tend to emphasize the inference, based upon the leaf structure, that the structure of the plant is that of a hygrophyte or mesophyte rather than of a xerophyte.

The leaf teeth bear hydathodes, which are differentiated very early. A longitudinal section shows that the bundle supplying the hydathode expands beneath a well-differentiated epithem, in which fine and tortuous intercellular passages communicate with the exterior by means of one or few water-pores. These constitute efficient safety valves when, after heavy rainfall, the immature leaf has to deal with a sudden intake of water, the escape of which by transpiration is more or less checked by the, as yet, close investment of hairs.

Floral morphology. The flowers usually begin to expand in this country early in April, but may be already differentiated in the rosette condition in February. The normal flowering period extends from April to June, flowers continuing to be formed whilst ripe achenes are being shed. In natural habitats, however, the flowering period is normally short, owing to the drying up of the plant. The first flower terminates the main axis, which always remains short. The axillary buds then develop, and these in turn end in a flower. Except for these primary branches, which may be of some length, the axillary buds usually give rise to an axis bearing only one leaf and a terminal flower. Hence each flower comes to occupy a position on the opposite side of the sympodial axis to a leaf. Each node thus appears to bear a leaf and a flower. The arrangement of the successive leaves on the sympodial inflorescence is approximately in a $1/3$ spiral, or sometimes even distichous, but displacements are caused by (*a*) mechanical pressure, (*b*) unequal development of the internodes, (*c*) formation of longer branches in place of axillary shoots bearing only one flower and one leaf. The main axis of the plant is always terminated quite early by a flower (cf. Pl. XVIII, Fig. 12), but the axillary buds produced from the axils of the upper rosette leaves may at first, especially under conditions of favourable nutrition, bear several leaves and axillary branches before terminating in a flower.

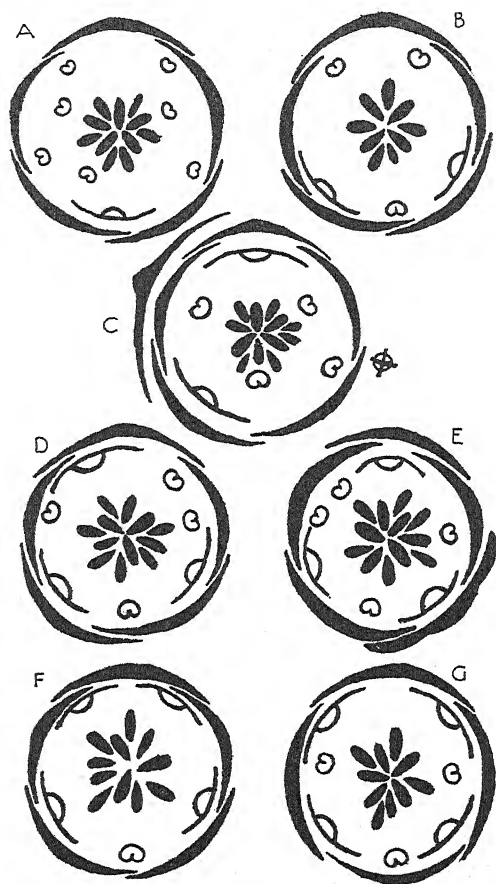
The flower. The development of the flower has been followed by means of series of microtome sections of specimens at various stages. These show that the flower does not exhibit throughout the normal acropetal succession. Sections of immature flower-buds exhibit stamens in the mature condition, whilst the petals are not fully developed. The sequence of development is: (1) sepals, (2) stamens, (3) petals, (4) carpels.

The nectary on each petal differentiates early, but the expansion of the petal lamina is delayed. This retarded development of the petals can be compared with the retarded development of the modified calyx in the Compositae, and may perhaps be regarded as an accompaniment of the phylogenetically recent modification. As will appear later, *Ranunculus parviflorus* affords clear evidence of the staminal origin of the petals, and here too the retarded development may well be a consequence of the recent modification which might, for example, be associated with a vascular supply in the enlarged staminode, hardly commensurate with its enhanced bulk.

Examination of seven hundred and twenty-five flowers in their entirety from a diversity of localities, and of numerous others with respect to one or other of the floral whorls, has furnished data that constitute a reliable picture of the range of variation which this species presents.

The calyx. The members of this whorl exhibit remarkable constancy, both as to arrangement and number. Only one example has been met with in which the number of sepals was less than five (Text-fig. 8, G), and none in which the sepals were in excess of that number. With this one exception, the sepals are always quincuncially arranged, and their constancy in this respect offers a striking contrast to the diversity in arrangement of the petals in some species of *Ranunculus*. The varied types of overlap in the corolla are indicative of the chance relations of space as the petals develop, so that no importance can attach to it. But the constancy of the manner of overlap in the calyx is significant and calls for explanation. Of these five sepals two are external, two internal, whilst the fifth has one margin internal and the other margin external. This sepal is further distinguished from the others by its asymmetrical form. This feature is even more marked in other members of the group (e.g. *Helleborus*), and must be attributed either to a direct outcome of its unique spatial relations, or to a different origin from the other members. The writer has already suggested that the quincuncial calyx is in reality a derivative from a condition with two whorls of three members each, consequent upon the congenital replacement of one member of the outer whorl and an adjacent member of the inner by a single member. Such a congenital fusion between an outer and an inner member would naturally result in the spatial relations which actually obtain, and according as the fusing inner member is on one side or the other, so we obtain the so-called 'right-handed' and 'left-handed' flowers, which occur together in the same inflorescence. If the spatial relations of sepals were determined by purely mechanical relations, then we should have the extreme variability in this respect which characterizes the corolla. That this is not the case argues that the spatial relations are determined by the phylogenetic history of this whorl. It is significant in this connexion that where, as in *Anemone*

nemorosa and *Helleborus*, both hexamerous and pentamerous calices are met with, the former present two whorls of three members each, whilst the latter are quincuncially arranged. In *Berberidaceae* both the quincuncial

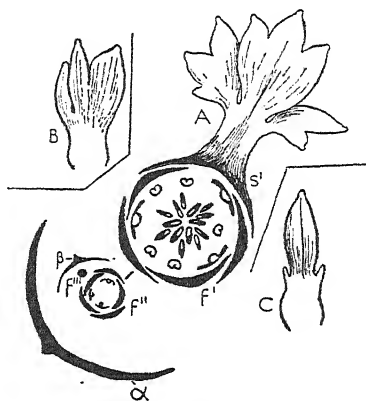


TEXT-FIG. 8. Empirical floral diagrams of seven out of the 116 types of floral organization observed. In C, D, and E (cf. also Text-fig. 10, F) the trimerous arrangement is obvious. A, B, and F illustrate types in which the tendency is towards a pentamerous arrangement. In both D and E the arrangement of parts indicate the replacement of a single stamen rudiment by two. The diagrams show clearly the interchangeability of petal and stamen.

and 3+3 arrangement are met with, according as the flower is lateral or terminal.

Although, with respect to number, the calyx exhibits such striking constancy, it is otherwise with respect to the form of its members. A careful study of the variations in sepal structure and its relation to that of the leaf at once suggests comparison with the leaf-base which, as already noted, is, like the sepals, membranous in character. In both sepal and leaf-base there are present three main bundles, sometimes with one or two

subsidiary strands totalling three to five bundles in all. The validity of the comparison is further shown when we consider the extreme variations of the sepals in which the normally membranous region is surmounted by a well-developed lamina, in every respect comparable to that of a normal leaf. In all, some thirteen flowers have been observed with one sepal foliaceous. As might be expected, this is normally the outermost member of the quincuncial spiral only, but one example possessed two such foliaceous sepals. These aberrant calices showed all degrees of development of the lamina from a small, simple, green apex of the otherwise membranous sepal (Text-fig. 10, B) to a fully differentiated lobed lamina, indistinguishable from that of a foliage leaf (Text-fig. 9, A). Moreover, in one sepal where a lamina was developed, the membranous portion had produced stipular lobes, comparable to those of the leaf-base (Text-figs. 9, C, and 10, A).



TEXT-FIG. 9. A. Floral diagram of flower with one sepal bearing a well-developed lamina (s'). B and C. Examples of less pronounced foliaceous sepals. Note the stipules in C.

If further evidence were necessary, it is furnished by a correspondence in physiological behaviour. The sepals are reflexed at maturity as the result of a greater extension towards the upper than towards the lower surface. In the leaf-base there is a similar epinastic curvature, in full illumination, as a consequence of which the leaves of the winter rosette are pressed against the soil (cf. Postscript, p. 576).

Thus the morphology, the anatomy, and the physiological behaviour of the sepals combine to emphasize the fact that they are in reality leaf-bases, modified to constitute bud-scales of the reproductive bud.

If a plant in the winter rosette condition be removed from the soil, the leaves at once bend downwards beyond the horizontal position, owing to the pronounced epinasty. On the other hand, in dull light the leaf-base exhibits no such epinasty, and the leaves tend to become more or less erect. It is interesting to note that the British species of *Ranunculus* with the most pronouncedly adpressed rosettes, viz. *R. parviflorus*, *R. bulbosus*, *R. hirsutus*, all possess calices with reflexed sepals, whilst in *R. repens* and *R. arvensis*, in which the sepals are spreading, the rosette habit is not nearly so pronounced. It is of interest to note that *R. acris*, which occupies a more or less intermediate condition as regards its rosette, has spreading sepals, but a strain with reflexed sepals has been recorded from various parts of Holland. The sepals are then to be regarded as the protective scales of the reproductive bud, which may or may not retain the

physiological characteristics of their prototype. The sepals of *Ranunculus* are thus comparable with the bud-scales of *Ribes*, *Aesculus*, or *Fraxinus*, both as to origin and function.

The corolla. The corolla consists of a varying number of petals; most frequently two (Text-fig. 8, B and C), sometimes five, occasionally lacking altogether. The percentage frequencies of the different conditions observed were as follows: No petals, 0.8 per cent.; one petal, 8.6 per cent. (Text-fig. 8, A); two petals, 47.4 per cent.; three petals, 40 per cent. (Text-figs. 8, D and E, and 10, F); four petals, 2.6 per cent. (Text-fig. 8, F and G); five petals, 0.4 per cent.

The normal occurrence of less than five petals was attributed by some of the older botanists to precocious shedding, but evidence in the form of scars has never been obtained to show that any such premature shedding takes place. Moreover, the low numbers of petals, and even their entire absence, has been noted in unopened buds as well as in expanded flowers.

In Table IV the correlation between the number of petals and the number of stamens is shown. The correlation is a negative one, namely, -0.36 , with a probable error of ± 0.0218 . The correlation is therefore definitely significant, and having regard to the spatial relations as between stamens and petals (Text-fig. 8) there can be no reasonable doubt that petals and stamens replace one another. Transitional forms between stamens and petals occur and serve to emphasize this conclusion. The petal is typically oval or elliptical in form, and consists of a short stalk about three-quarters of a mm. in length, bearing a lamina about 3 to 4 mm. long by 2 mm. in width (Text-figs. 11, A and 10, C).

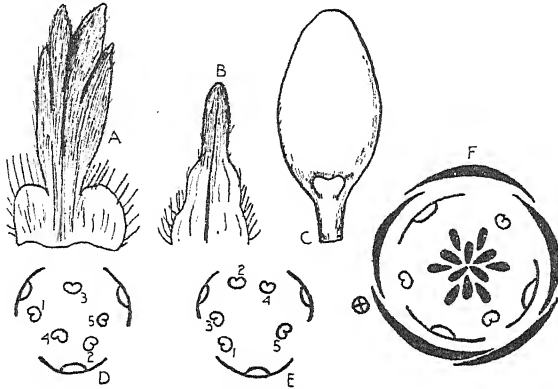
TABLE IV.

Correlation between Stamen Number and Petal Number in R. parviflorus.

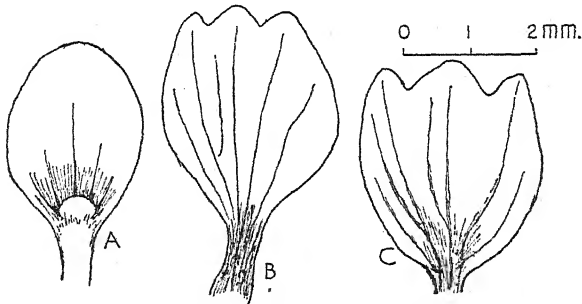
Stamen number.	Petal number.						Totals.
	0.	1.	2.	3.	4.	5.	
1	—	1	2	1	1	—	5
2	—	—	—	5	2	2	9
3	—	—	19	26	6	—	51
4	—	12	62	65	8	—	147
5	5	8	107	181	2	1	304
6	—	19	149	7	—	—	175
7	—	23	2	5	—	—	30
8	1	—	3	—	—	—	4
Totals	6	63	344	290	19	3	725
Correlation coefficient					-0.36		
Probable error					0.0218		

The lamina is of the glossy type (cf. Parkin (13)) and bears a saucer-shaped nectary on the inner face at the junction of stalk and limb. The

petal is supplied by a single trace which passes to the base of the nectary, where it expands beneath the secretory tissue (Pl. XVIII, Fig. 4); from this expansion five slender strands are given off which pass into the limb of the petal. Two examples were met with where the petal bore no nectary, and



TEXT-FIG. 10. A. Foliaceous sepal showing stipular appendages. B. Sepal with rudimentary lamina (green parts shaded in A and B). C. Petal with lobed scale. D and E. Empirical diagrams showing the order of dehiscence of the stamens in two flowers indicating their development in a $1/3$ spiral. F. Empirical diagram of a flower showing a $1/3$ spiral arrangement of parts throughout.

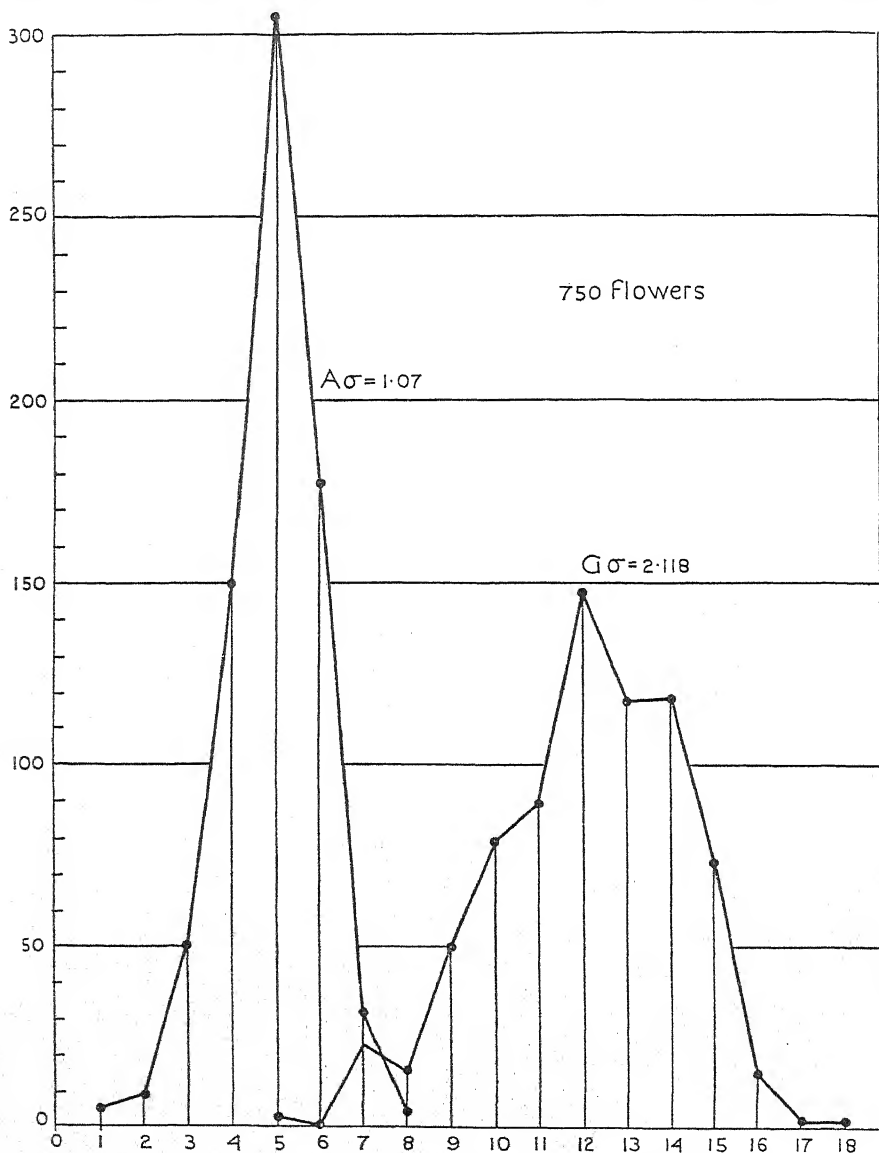


TEXT-FIG. 11. A. Normal petal. B and C. Lobed petals showing an augmented vascular supply.

the limb in both instances was slightly three-lobed at the apex. In both these petals there were more than the normal number of strands (Text-fig. 11, B and C).

Although the development of the petal as a whole is delayed, the nectary is differentiated at an early stage and is already, before the flower opens, capable of functioning as an osmotic hydathode. In relation to this the vascular supply embracing the secretory tissue is significant, and it is noteworthy, therefore, that the stamens will dehisce in an almost saturated atmosphere, doubtless due to the removal of water by the nectaries. The nectary is protected in front by a small scale, which may be entire (Text-fig. 11, A) or slightly bi-lobed (Text-fig. 10, C).

The androecium. As already stated, the stamens show a negative correlation with the petals, so that when the number of the latter is high



TEXT-FIG. 12. Meristic variation curves for the number of stamens and carpels in 750 flowers. Ordinates=number of examples, Abscissae=number of parts.

the number of stamens tends to be low. The observed range in number of stamens is from one to eight (Text-fig. 8, A and F).

The mode for the number of stamens is five, with a standard deviation of 1.07. The variation curve is shown in Text-fig. 12. Compared with

other members of the Ranunculaceae, which have been investigated from this point of view, the range in number of stamens is small. It is perhaps partly owing to the high proportion of flowers having the modal number of stamens that there is little, if any, indication of secondary modes. A striking feature of the staminal variation in several members of the Ranunculaceae investigated by the writer, viz. *Eranthis hyemalis*, *Ficaria verna*, *Anemone apennina*, &c., was the periodic nature of the variation curves, which not only exhibited a primary mode corresponding to a multiple of three, but also secondary modes likewise corresponding to multiples of three (16, especially Figs. 7 and 12, and 17, Fig. 3). The androecium of *R. arvensis* was investigated by Burkill (3) from this point of view, and although this species has a range in number of stamens of from one to eighteen, the same absence of secondary modes is to be noted as shown in Text-fig. 13, which has been constructed from Burkill's data. If, as the writer suggests, the trimerous organization is a primitive character, then in this respect these two species of *Ranunculus* exhibit what is perhaps a lack of specialization as compared with members of other genera, and even as compared with some other species of *Ranunculus* which the writer has examined. For in these two species all the parts in the flower must be considered as a whole, whereas in those species in which the androecium shows a periodic curve of meristic variation the floral whorls vary independently.

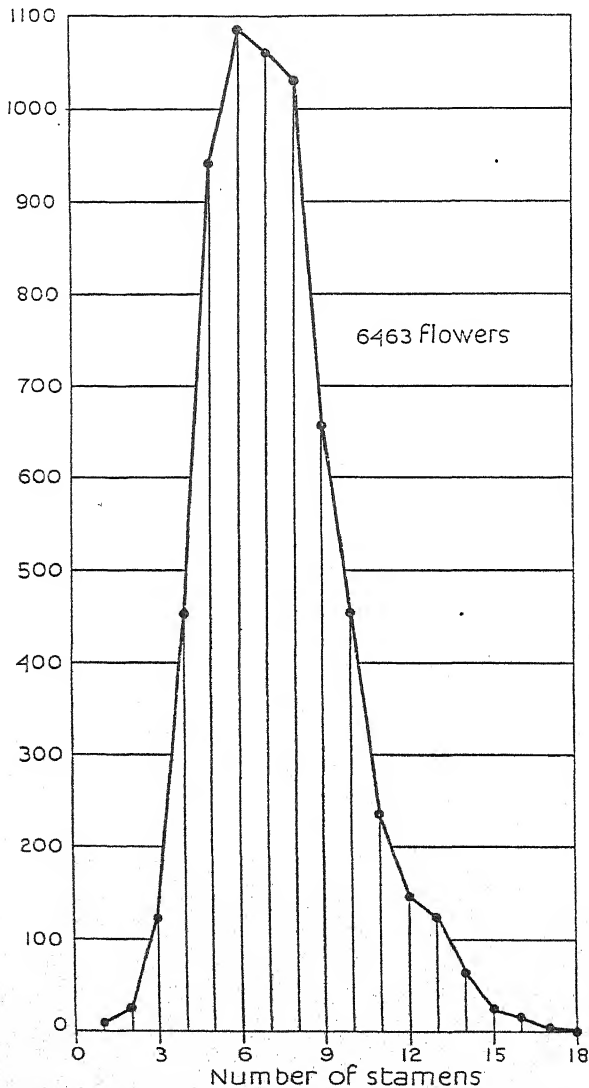
The individual stamen when young shows a differentiation of the products of division of the primary wall-cell into three layers. The outermost becomes a fibrous endothecium, the second forms a middle layer of narrow cells, and the innermost becomes a conspicuous tapetum of large cells, sometimes with more than one nucleus.

Dehiscence is extrorse, and is probably brought about normally by desiccation in the usual manner, but in very moist air there can be little doubt that the nectaries, functioning as osmotic hydathodes, bring about dehiscence by withdrawal of water from the stamen. Stamens containing numerous mature elliptical pollen-grains are already present in the flower a considerable time before it opens (Pl. XVIII, Fig. 12).

No instance of a branched stamen has been seen, although such are not infrequent in other Ranunculaceae (cf. Salisbury, 16 and 17), but on the other hand the position and arrangement of the stamens in some flowers certainly suggests congenital replacement of a single staminal rudiment by two. This, for example, is probably the explanation of the paired stamens in the flowers represented in the Diagrams D and E of Text-fig. 8, and may be compared with the similar phenomenon in *E. hyemalis*.

The gynoceium and ovule. The carpels of *R. parviflorus* exhibit several interesting features. At maturity they bear curved or hooked hairs which

are stiff, and possibly assist in fruit dispersal by the agency of animals, although it is probable that water-transport in rain-wash may be as effective



TEXT-FIG. 13. Meristic variation in the androecium of *Ranunculus arvensis*, based on the data of I. H. Burkill, showing a mode of six.

or even more so than dispersal by animals, for which latter there would appear to be the more adequate provision. The curved bristle-like hairs are developed at an early stage, being already conspicuous in the flower-bud.

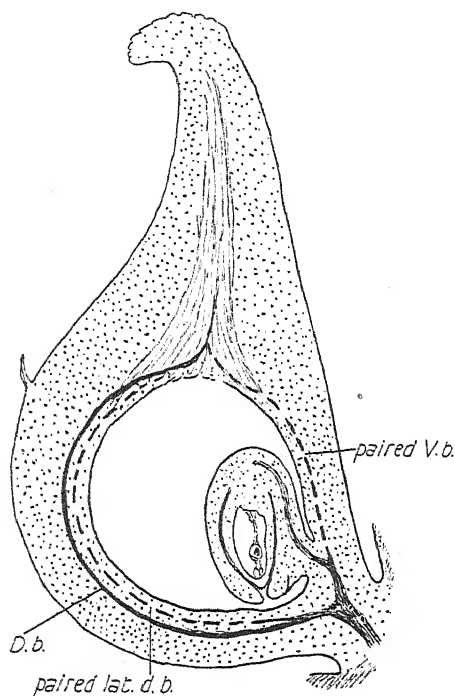
Each carpel in the very young stage appears as an oval rudiment,

convex below and concave above, with a swelling in its axil (*ov.*, Text-fig. 15). The comparison of the carpel with a young leaf-rudiment is at once suggested, and the axillary protuberance, which later becomes the ovule, appears to strengthen this comparison. This rudiment as it enlarges curves upwards and inwards, and becomes boat-shaped with a cucullate apex (Text-fig. 15, C); finally the margins meet and fuse above so as to completely enclose the solitary ovule. The carpel when fully developed is supplied with a single vascular strand from the receptacle, which soon divides in the vertical plane to form two bundles, one of which (the so-called dorsal bundle (Text-fig. 14, *D.b.*)) is the midrib of the carpellary leaf, and passes up into the apex of the carpel, whilst the other branch divides into two strands, which traverse the margins of the carpel parallel to one another (paired ventral) (Text-fig. 14 *V.b.*). The ovular trace is either given off simultaneously with the two marginal traces or more usually it is derived from one or other of the marginal strands, thus showing that the ovule is actually marginal in origin despite its apparent axillary position. The dorsal bundle gives off a lateral strand on either side, soon after it separates from the ventral branch, and these two strands pass along the dorsal margin of the carpel parallel to, and on either side of, the median dorsal vein. The mature carpel in transverse section thus presents five vascular strands, namely the median dorsal, the two-paired lateral dorsal veins, and the two-paired ventral veins. When the ovular trace is given off at the same level as that at which the ventral veins diverge, the main ventral bundle appears to trifurcate, and the ovule may be said to be apparently if not actually axillary but, as already noted, the origin of the ovular trace from one of the ventral strands after their separation (marginal veins of the carpellary leaf) is sometimes obvious (Pl. XVIII, Fig. 5).

The general vascular organization of the carpel is almost the same as that described by Chute (4) for *R. repens*, though in respect to the origin of the ovular trace from a lateral ventral strand *R. parviflorus* approaches more nearly the condition described by Chute for *R. acris*, in which the origin of the ovular trace from one of the ventral traces after their separation is apparently the rule. As Chute points out, this organization emphasizes the origin of the achene from a follicle, and it is noteworthy that in *F. verna* which, according to Chute, approaches still closer in vascular supply to the folliculate condition, two ovules in an achene have been recorded by Masters (11), and the staminal variation, as shown by the present writer, exhibits a marked periodicity with modes corresponding to multiples of three (cf. Salisbury (16)).

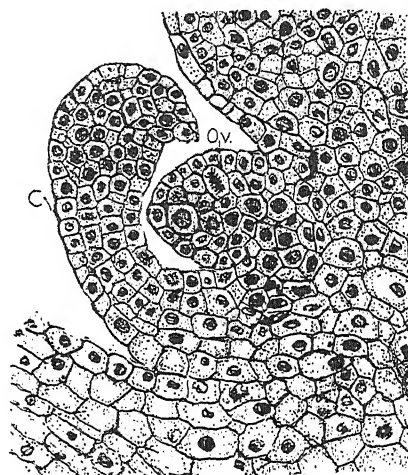
The species of *Anemone*, many of which have been examined from this point of view by the author, exhibit varying degrees of retention of abortive ovules indicating the origin of the achene in this genus from a multiovulate follicle. In the most extreme instances only a single

abortive ovule is present, or even this may not develop. It is therefore of interest to note that in *R. parviflorus* a slight, and otherwise apparently meaningless, swelling is present in the young ovary in a position which



TEXT-FIG. 14.

TEXT-FIG. 14. Diagram of a longitudinal median section of a carpel to show the vascular system. *D.b.* Dorsal bundle. The positions of the lateral dorsal bundles (paired lat. *d.b.*) and the ventral bundles (paired *V.b.*), both of which are paired and therefore not in the median plane, are indicated by broken lines.



TEXT-FIG. 15.

TEXT-FIG. 15. Median longitudinal section through a very young carpel (*c.*) and ovule (*ov.*) showing a unicellular archesporium. The apparently axillary position of the ovule is well seen, and the carpel has not yet closed in above.

suggests it may represent the last vestige of the rudiment of a second and abortive ovule (Pl. XVIII, Figs. 2 and 4).

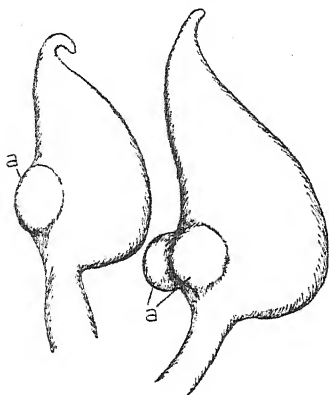
The ovule itself presents certain interesting features with respect to its form and development. The archesporium differentiates in the nucellus before the single integument is more than an asymmetrical enlargement of the ovule. Usually it would appear that the archesporium consists of a single cell (Pl. XVIII, Fig. 3). Occasionally, however, the archesporium consists of several cells (Pl. XVIII, Fig. 6); thus our species recalls the condition described by Coulter for *R. septentrionalis* (in which also both unicellular and multicellular archesporia occur (5). Coulter and Chamberlain (6), after reviewing the observations of various investigators on the Ranunculaceae, conclude: 'It is evident, therefore, that the Ranunculaceae,

while ordinarily producing a one-celled archesporium, show a strong tendency to an increase in the number of cells.' If, however, despite the occurrence of multicellular archesporia in various Sympetalous species, this condition is, as it well may be, primitive, then the last part of the paragraph just quoted should read . . . show a strong tendency to a reduction in the number of cells. Multicellular archesporia have been recorded from various folliculate members of the group, and are perhaps more frequent amongst these than in those members of the group in which the follicle has been reduced to an achene. Certainly in one other respect the histology of the ovule of *R. parviflorus* departs from the normal condition in the genus, and in a respect which is undoubtedly more primitive. Coulter and Chamberlain (6, p. 64) state that 'the Ranunculaceae exhibit a surprisingly uniform suppression of the parietal tissue, this condition having been found in *Anemone*, *Caltha*, *Clematis*, *Delphinium*, *Myosurus*, and *Ranunculus*; while in *Aquilegia* a parietal cell may or may not appear.' The ovule of which a median section is shown in Pl. XVIII, Fig. 3, shows a megaspore mother-cell which has undergone the first division, and between these and the epidermis a primary parietal cell which has divided into two by a vertical wall. It is interesting to note that the periclinal divisions in the nucellar epidermis, which Coulter figures in *R. septentrionalis* just above the archesporial cell, occur also in *R. parviflorus*, but in the example figured (Pl. XVIII, Fig. 3) are absent from the cells immediately over the parietal cells. Whether the production of primary parietal cells by *R. parviflorus* is of general occurrence or not, the available material in an appropriate stage of development does not permit of answering. However, it is significant that here, even if only occasionally, division of the archesporial cell into a primary parietal and primary sporogenous cell does occur, and that multicellular archesporia may also be present.

At first the ovule is typically anatropous (Pl. XVIII, Fig. 4), but after fertilization the ovule and embryo-sac enlarge in such a manner that the structure is definitely amphitropous (Pl. XVIII, Fig. 1). In view of the fact that typically amphitropous ovules pass through an anatropous phase in their ontogeny, this post-fertilization development is of some interest.

Already, before fertilization, the ovule only occupies a part of the cavity of the carpel, and even by the time the achene has attained maturity the contained seed does not fill the space completely. As a consequence the achenes float readily, and for this reason are suited for dispersal by rain-wash. The form of the carpels is fairly constant, although variations in the length of the beak are not uncommon. Two instances have been met with in which a young carpel bore definite anther lobes containing pollen-grains (Text-fig. 16). Such instances afford evidence of the absence of any hard and fast boundary between the rudiments of one type of whorl and those of the whorls adjacent. Moreover, the lack of demarcation

between stamens and petals already noted, their negative correlation, and the corresponding transitions between carpels and stamens, shows that the



TEXT-FIG. 16. Two carpels bearing anther lobes (a.) which contained pollen.

whorls must be considered together if we are to understand their relationship to rudiment-production at the growing apex. Since structures that combine the characters of both stamen and carpel may occur, it is reasonable to suppose that, though in general a whorl of the spiral succession will consist of a single type of structure, yet that occasionally the one type may replace the other more or less completely.

Meristic variation in the gynoecium.

The observed number of carpels ranges from three to eighteen, with twelve as the mode (Text-figs. 12 and 17).

The number of carpels formed in the flower varies not only with the vigour of the plant, but also with the period of flower formation, both perhaps an outcome of nutritional factors.

In the following table (Table V) are given the number of carpels in successive flowers on different branches of a luxuriant plant, in order from below upwards. Altogether twenty-five branches were examined and, although the total number of flowers (175) is rather small, yet it is obvious from the positions of the modes for carpel number at each level, and the average number of carpels, that there is a definite tendency for the number of carpels to diminish with the progress of the flowering season. This same feature was observed in plants where, owing to the unfavourable conditions in the early part of the growing season, a considerable proportion of the carpels aborted. This suggests that the factors, if nutritional, are not merely a simple relation between food supply and carpel number. The direct effect of competition upon the formation of achenes is best illustrated by comparison of the carpel numbers in flowers of robust and depauperate starved plants in a crowded population. In Text-fig. 17 are shown the variation curves for carpel number in 175 flowers borne by starved plants, and in 175 flowers borne by robust plants growing mingled together. It will be noted that the mode for the robust plants is thirteen, whilst for the starved plants it is nine. Moreover, the range for the latter is only three to twelve, whilst for the former it is nine to seventeen.

In Table VI is shown the correlation between the number of carpels and the number of stamens. The correlation is a negative one, and though slight, namely -0.062 with a probable error of ± 0.0249 , and therefore perhaps not significant, it is not improbable that the marked protandry of

this species might result in a slight diminution of the number of carpels when the androecium consists of seven or eight stamens.

TABLE V.

Relation of Carpel Number to Position on Inflorescence.

Number of flower in order on branch from below.	Number of carpels in each flower (Plant Y).										Totals.	Average.
Lowest flowers.	9.	10.	11.	12.	13.	14.	15.	16.	17.			
1st.	—	—	—	3	3	5	4	8	2	25	14.6	
2nd.	—	—	—	2	7	4	8	3	1	25	14.2	
3rd.	—	—	1	0	10	7	7	0	—	25	13.8	
4th.	—	—	1	5	9	6	3	1	—	25	13.3	
5th.	—	—	3	10	7	3	1	1	—	25	12.7	
6th.	1	1	5	4	8	4	2	0	—	25	12.5	
7th.	1	0	4	10	3	4	3	0	—	25	12.5	
	2	1	14	34	47	33	28	13	3	175		

TABLE VI.

Correlation between Stamens and Carpels.

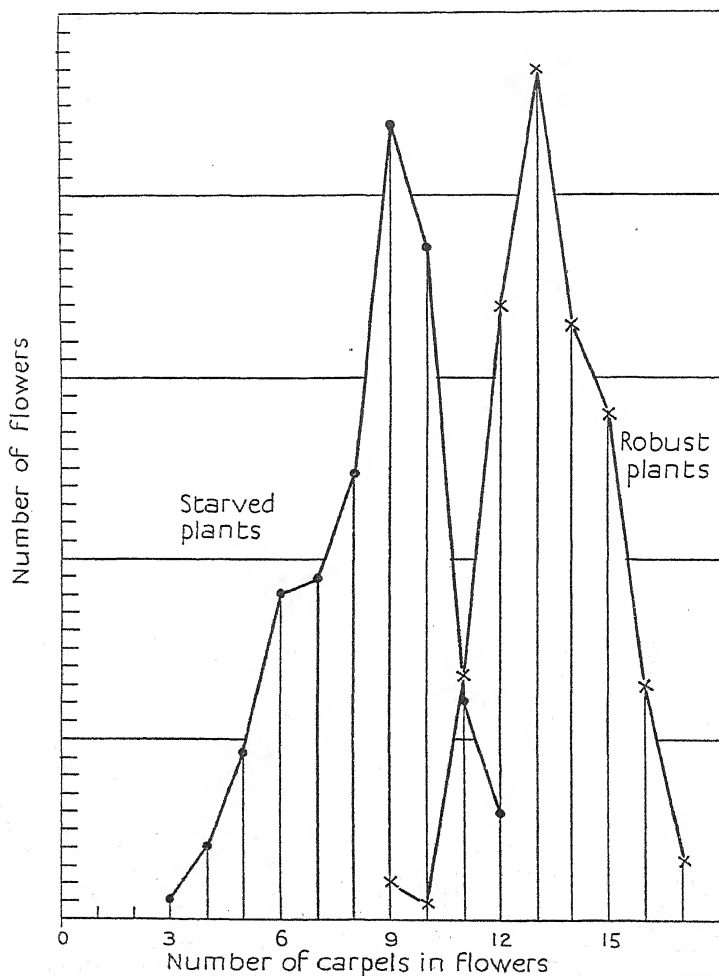
Correlation coefficient -0.062 ± 0.0249 .

Number of carpels.	Number of stamens.								Totals.
	A 1.	A 2.	A 3.	A 4.	A 5.	A 6.	A 7.	A 8.	
G. 5	—	—	—	—	2	—	—	—	2
G. 6	—	—	—	—	—	—	—	—	0
G. 7	—	—	—	6	11	4	—	—	21
G. 8	—	—	—	4	8	3	—	—	15
G. 9	—	—	3	15	14	16	1	—	49
G. 10	—	1	3	17	33	20	4	—	78
G. 11	—	—	7	21	32	24	3	2	89
G. 12	3	2	15	32	54	31	8	2	147
G. 13	—	—	6	16	51	35	9	—	117
G. 14	—	1	9	18	61	27	2	—	118
G. 15	—	4	7	15	29	14	3	—	72
G. 16	1	1	1	1	9	1	—	—	14
G. 17	—	—	—	2	—	—	—	—	2
G. 18	1	—	—	—	—	—	—	—	1
Totals	5	9	51	147	304	175	30	4	725

It is evident then that, apart from a possible very slight relation to the androecium, the gynoecium behaves as an independent unit in the flower, affected mainly by conditions of nutrition. It is, therefore, of considerable interest to note that it is this part of the flower that exhibits trimery in the most marked degree. Sections through very young flower buds show quite clearly that the carpels are usually formed in a spiral succession, three carpels being present in each whorl, alternating with those previously formed (cf. Pl. XVIII, Fig. 8). The mechanical displacement of the carpel

rudiments is also clearly evident, and explains the departures which are to be observed in the mature flower.

If we turn now to consider the structure of the flower as a whole, we



TEXT-FIG. 17. Meristic variation curves for the number of carpels in 175 robust plants and 175 starved plants, the mode for the former being thirteen and for the latter nine. Ordinates = number of flowers, Abscissae = number of carpels.

find that no less than one hundred and sixteen types of floral organization have been met with.

They are as follows :

K 4, C 4, A 3, G 10 (1)	K 5, C 0, A 5, G 5 (2)	K 5, C 0, A 5, G 8 (2)
K 5, C 0, A 5, G 15 (1)	K 5, C 0, A 8, G 12 (1)	K 5, C 1, A 1, G 12 (1)
K 5, C 1, A 4, G 7 (4)	K 5, C 1, A 4, G 8 (2)	K 5, C 1, A 4, G 9 (2)
K 5, C 1, A 4, G 11 (4)	K 5, C 1, A 5, G 8 (2)	K 5, C 1, A 5, G 10 (2)

K 5, C 1, A 5, G 13 (2)	K 5, C 1, A 5, G 15 (2)	K 5, C 1, A 6, G 7 (2)
K 5, C 1, A 6, G 8 (2)	K 5, C 1, A 6, G 9 (3)	K 5, C 1, A 6, G 11 (7)
K 5, C 1, A 6, G 12 (3)	K 5, C 1, A 6, G 13 (1)	K 5, C 1, A 6, G 15 (1)
K 5, C 1, A 7, G 10 (4)	K 5, C 1, A 7, G 11 (3)	K 5, C 1, A 7, G 12 (8)
K 5, C 1, A 7, G 13 (5)	K 5, C 1, A 7, G 14 (2)	K 5, C 1, A 7, G 15 (1)
K 5, C 2, A 1, G 12 (2)	K 5, C 2, A 3, G 9 (3)	K 5, C 2, A 3, G 10 (2)
K 5, C 2, A 3, G 11 (5)	K 5, C 2, A 3, G 12 (4)	K 5, C 2, A 3, G 14 (4)
K 5, C 2, A 3, G 16 (1)	K 5, C 2, A 4, G 7 (2)	K 5, C 2, A 4, G 8 (2)
K 5, C 2, A 4, G 9 (12)	K 5, C 2, A 4, G 10 (10)	K 5, C 2, A 4, G 11 (11)
K 5, C 2, A 4, G 12 (10)	K 5, C 2, A 4, G 13 (6)	K 5, C 2, A 4, G 14 (4)
K 5, C 2, A 4, G 15 (4)	K 5, C 2, A 4, G 16 (1)	K 5, C 2, A 5, G 7 (8)
K 5, C 2, A 5, G 8 (2)	K 5, C 2, A 5, G 9 (13)	K 5, C 2, A 5, G 10 (23)
K 5, C 2, A 5, G 11 (13)	K 5, C 2, A 5, G 12 (15)	K 5, C 2, A 5, G 13 (9)
K 5, C 2, A 5, G 14 (17)	K 5, C 2, A 5, G 15 (5)	K 5, C 2, A 5, G 16 (2)
K 5, C 2, A 6, G 7 (2)	K 5, C 2, A 6, G 8 (1)	K 5, C 2, A 6, G 9 (13)
K 5, C 2, A 6, G 10 (20)	K 5, C 2, A 6, G 11 (17)	K 5, C 2, A 6, G 12 (25)
K 5, C 2, A 6, G 13 (33)	K 5, C 2, A 6, G 14 (24)	K 5, C 2, A 6, G 15 (13)
K 5, C 2, A 6, G 16 (1)	K 5, C 2, A 7, G 9 (1)	K 5, C 2, A 7, G 13 (1)
K 5, C 2, A 8, G 11 (2)	K 5, C 2, A 8, G 12 (1)	
K 5, C 3, A 1, G 16 (1)	K 5, C 3, A 2, G 12 (1)	K 3, C 3, A 2, G 14 (1)
K 5, C 3, A 2, G 15 (2)	K 5, C 3, A 2, G 16 (1)	K 5, C 3, A 3, G 11 (2)
K 5, C 3, A 3, G 12 (10)	K 5, C 3, A 3, G 13 (4)	K 5, C 3, A 3, G 14 (4)
K 5, C 3, A 3, G 15 (6)	K 5, C 3, A 4, G 9 (1)	K 5, C 3, A 4, G 10 (7)
K 5, C 3, A 4, G 11 (6)	K 5, C 3, A 4, G 12 (21)	K 5, C 3, A 4, G 13 (8)
K 5, C 3, A 4, G 14 (13)	K 5, C 3, A 4, G 15 (8)	K 5, C 3, A 4, G 17 (1)
K 5, C 3, A 5, G 7 (3)	K 5, C 3, A 5, G 8 (2)	K 5, C 3, A 5, G 9 (1)
K 5, C 3, A 5, G 10 (8)	K 5, C 3, A 5, G 11 (19)	K 5, C 3, A 5, G 12 (38)
K 5, C 3, A 5, G 13 (40)	K 5, C 3, A 5, G 14 (44)	K 5, C 3, A 5, G 15 (21)
K 5, C 3, A 5, G 16 (5)	K 5, C 3, A 6, G 12 (3)	K 5, C 3, A 6, G 13 (1)
K 5, C 3, A 6, G 14 (3)	K 5, C 3, A 7, G 13 (3)	K 5, C 3, A 7, G 15 (2)
K 5, C 4, A 1, G 18 (1)	K 5, C 4, A 2, G 15 (2)	K 5, C 4, A 3, G 12 (1)
K 5, C 4, A 3, G 13 (2)	K 5, C 4, A 3, G 14 (1)	K 5, C 4, A 3, G 15 (1)
K 5, C 4, A 4, G 12 (1)	K 5, C 4, A 4, G 13 (2)	K 5, C 4, A 4, G 14 (1)
K 5, C 4, A 4, G 15 (3)	K 5, C 4, A 4, G 17 (1)	K 5, C 4, A 5, G 15 (2)
K 5, C 5, A 2, G 10 (1)	K 5, C 5, A 2, G 12 (1)	K 5, C 5, A 5, G 12 (1)

The most frequent types of construction have five sepals, three petals, and five stamens, with from twelve to fourteen carpels. Such comprise 16.8 per cent. of the total examined. Next in frequency are flowers with five sepals, two petals, six stamens, and with from twelve to fourteen carpels, which comprise 11.3 per cent. Since, as we have seen, petals and stamens show a considerable negative correlation, one may regard flowers with two petals and six stamens as equivalent to those with three petals and five stamens, so that the condition with eight members in these two whorls together is by far the most frequent.

Inasmuch as the flower is a terminal structure, and represents a reproductive bud in its entirety, the total number of parts might be expected to give some indication as to whether or no there is a tendency for the growing point to form lateral members in groups of threes. A study of the variation curve for the total number of parts (Text-fig. 18) and the data in Table VII show that these totals range from fifteen as the lower

limit to thirty as the upper, with a pronounced mode at twenty-six. If we accept the view that the calyx represents six members, of which two are congenitally fused, then, if the remaining turns of the spiral consist of three members each, we should expect a total which is one less than a multiple of three. The mode of twenty-six thus fulfils these expectations, and supports our assumption that the growing point tends to produce the lateral members in complete turns of the spiral of three members each.

TABLE VII.

Correlation between Number of Carpels and Total Number of Parts in the Flower.

Total parts.	Number of carpels.														
	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	
15	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
17	—	—	4	—	—	—	—	—	—	—	—	—	—	—	4
18	—	—	2	4	—	—	—	—	—	—	—	—	—	—	6
19	—	—	10	4	5	—	—	1	—	—	—	—	—	—	20
20	—	—	5	4	12	2	—	2	—	—	—	—	—	—	25
21	—	—	—	3	17	13	9	—	—	—	—	—	—	—	42
22	—	—	—	—	14	31	13	5	—	—	—	—	—	—	63
23	—	—	—	—	1	39	19	20	—	—	—	—	—	—	79
24	—	—	—	—	—	—	39	41	12	5	—	—	—	—	97
25	—	—	—	—	—	—	—	72	20	8	3	1	—	—	105
26	—	—	—	—	—	—	2	3	80	31	14	2	—	—	132
27	—	—	—	—	—	—	—	2	2	71	15	1	—	—	91
28	—	—	—	—	—	—	—	—	3	3	38	2	—	1	47
29	—	—	—	—	—	—	—	—	—	—	2	7	—	—	9
30	—	—	—	—	—	—	—	—	—	—	2	—	1	—	3
Totals.	2	0	21	15	49	85	82	147	117	118	74	13	1	1	725

Arithmetic mean for total number of parts 24.4. Standard deviation 2.48.

Arithmetic mean for number of carpels 12. Standard deviation 2.118.

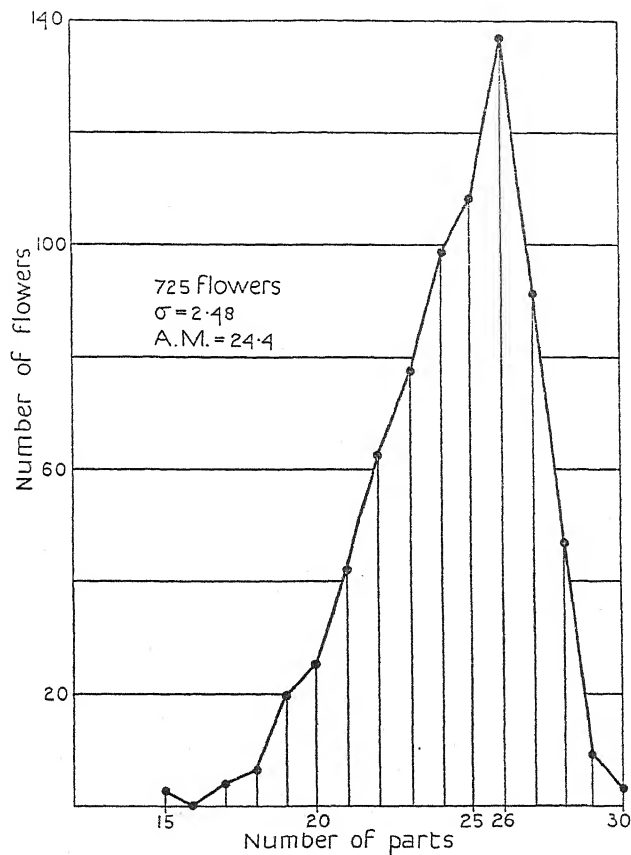
Correlation coefficient between total parts and number of carpels 0.95; probable error 0.01214.

An examination of the relation between total number of parts and carpel number shows a very high degree of correlation between them, the correlation coefficient being +0.95, and the probable error 0.01214. Clearly, then, the variation in total number of parts is mainly dependent upon the variation in the number of carpels, as might be anticipated from the probably non-significant correlation between stamen number and carpel number.

What has already been advanced in support of the view that conditions of nutrition govern the number of carpels, therefore, probably applies also to the total number of parts in the flower.

Transverse sections through very young flower buds (Pl. XVIII, Fig. 8) often show quite plainly that all the parts are formed in a spiral succession

of three members in each completed whorl, which alternate with those formed previously. In the specimen figured there are nine carpels, five stamens and one petal, forming a regular sequence of this type. In Text-fig. 3, is shown a section of a more advanced flower bud in which, of the



TEXT-FIG. 18. Variation curve for total number of parts in 725 flowers. Ordinates = number of flowers, Abscissae = total number of parts.

six stamens present, three inner alternate with the three outer, and the uppermost carpels to the number of three just come into the plane of section.

Seed output. The total number of achenes produced was counted for a number of normal plants, and estimated for some of the largest. A single exceptionally luxuriant specimen produced no less than 494 fruit-heads, and the average number of carpels based on counts of fifty fruit-heads selected at random was 13.6. Thus the total output of achenes was about 6,700. It will, however, be noted that the average number of carpels was

in this luxuriant specimen higher than the mean for a normal population, which is twelve. The following complete counts of the achenes produced by twelve specimens selected at random from a wild population, growing in the poor soil, which this species commonly frequents, gives a better picture of the normal reproductive capacity. The average number of achenes is 246; sometimes these wild plants are very depauperate, and two specimens were observed with only seven and fifteen achenes respectively. Two specimens from an arable field produced respectively 2,497 achenes and 674.

TABLE VIII.

Number of Achenes produced by Wild Plants.

Plant A	763	Plant E	286	Plant I	93
" B	537	" F	135	" J	93
" C	369	" G	128	" K	81
" D	323	" H	94	" L	54

It is evident, therefore, that the normal production of carpels does not usually exceed about 250, but that both size of plants and the output of fruits indicate that *Ranunculus parviflorus* grows better on soils other than the poor ones that it frequents in its natural habitats.

Germination. The achenes usually ripen and are shed from the middle of May to the middle of July, according to the character of the weather.

If early maturing achenes are sown as soon as they become ripe, they fail to germinate, and this applies equally to fruit which ripens towards the end of one season (i.e. in July). A striking illustration of this is afforded by seeds collected from a plant on June 8, and sown immediately under conditions known to be favourable for the germination of this species. After six weeks not a single achene had germinated. The achenes were then allowed to dry off, and again placed to germinate under similar conditions on September 21. A week later every seed had germinated. These and similar experiments in the laboratory confirmed the conclusion, based on field observations, that the achenes shed at whatever time from May to July all germinate about the same period, but at no other time of the year.

Achenes which are stored beyond the normal period will germinate at other times when placed under suitable conditions, and achenes which are artificially buried by the plough, or naturally by earthworms, may, when again brought to the surface, germinate perhaps at abnormal seasons. There is, however, no definite evidence of this beyond one or two records of plants found coming into bloom in the autumn which may be due to such induced retardation. In general, however, it may be said that all

viable achenes germinate about the same time, and that those which fail to germinate in the early autumn are non-viable.

R. parviflorus is thus a very striking example of a species exhibiting delayed germination, and belongs to the class we may designate 'Winter annuals'. Moreover, unlike some members of this category, it does not sometimes behave as a spring annual also.

It might be thought that this retarded germination was due to inadequate development of the embryo at the time of shedding since, as in so many Ranunculaceae, the embryo is ill-differentiated in the so-called resting fruit. But even if this be in part true, that it is not the complete explanation is indicated by the simultaneous germination of achenes shed early and late, despite the five or six weeks' interval between their shedding.

A close study of the germination, both in the field and laboratory, reveals that the majority of achenes germinate together, and that rarely is there an interval of more than three or four days between the first and most belated. *R. parviflorus* is therefore a marked example of 'simultaneous germination' (21).

The percentage germination is normally high, and sometimes attains 100 per cent. Fruits which had been kept for five years still remained in part viable with a percentage germination of 19.6. The effect of such storage on the viable achenes was to bring about a discontinuity of germination. The achenes which were sown at the end of September germinated as under.

Date.	%.	
16.10.30.	9.5	
17.10.30.	+3.3	Total 12.8
18.10.30.	+2.7	" 15.5
26.10.30.	+2.7	" 18.2
5.11.30.	+1.4	" 19.6

It will be seen that even here 79 per cent. of the viable seeds germinated within three days, followed by 14 per cent. and 7 per cent. at intervals of eight and ten days respectively.

The seedlings attain the rosette condition in the late autumn, and in this state persist during the winter months. The only exception to this generalization which has been encountered is a specimen in the British Museum Herbarium, collected by Augustine Ley in a turnip field in Brecon, and bearing the date September 25. Either this represents a precociously germinating achene, from which a plant developed sufficiently early to flower before the autumn drop in illumination and temperature, or more likely the plant in question originated from a buried achene of a previous season.

The simultaneous mode of germination which is here so marked has

its advantages for competition, but marked dangers under catastrophic disasters. Thus in the severe winter of 1928-29, when the minimum temperature was 12° F., all the seedlings of this species in the writer's garden were destroyed, and as no later germinations occur the species thus became extinct. Druce in the 'Flora of Oxfordshire', published in 1886, comments on the marked decrease of *R. parviflorus* in that county during the preceding half century (7). The writer is indebted to Mr. Richard Marshall for permitting him to consult his analysis of the records of winter temperatures during the past 200 years. These show that during the period referred to by Druce (1836-86), not only were there an exceptional number of hard winters, but several of these were of extreme severity, notably 1837-8, 1840-1, 1844-8, 1860-1, and 1866-7, with minimum temperatures respectively of -4°·5 F, 4°, 7°·7, 8°, and 6°·6 F, at Greenwich.

The susceptibility to frost is in harmony with the distribution of the species both in Britain and in Europe generally.

Geographical Distribution and Ecology.

R. parviflorus, as a wild species, has its northern limit in Britain (Durham) and Western France. Southwards it extends to Spain and Portugal northern Africa, Algiers, Morocco, and eastwards along the Mediterranean seaboard to Southern Greece, Thrace, Taurida, and Cyprus. It also occurs in the Canaries, Madeira, and the Azores. It is absent from northern and central Europe, and is rare or absent in eastern France.

The general distribution of the species in Europe is thus typically atlantic in character (Text-fig. 19) and associated with an oceanic climate. This feature is further emphasized by the greater frequency, both in France and Britain, towards the western seaboard in contrast with its increasing rarity as one approaches the eastern limit (Text-figs. 19 and 20).

In England *R. parviflorus* probably attains its maximum frequency in the Cornish peninsula, and in south Devon may be the most abundant weed in certain arable fields.

Bentham, in the 'Flora Australiensis' (3, p. 14), states that the var. *australis* of the species (which only differs in the smaller flowers and achenes, and more frequently sessile flowers) occurs in water-holes on the tops of the ranges in Queensland, in moist pastures (cf. also Bailey (1), p. 8), and banks of rivers and lagoons in New South Wales and Victoria.

Introduced into New Zealand, where it was first recorded by Hooker in 1864, *R. parviflorus* there flowers from October to December. According to Cheesman it is abundant in pastures and waste places in both islands, but is not common in Otago and Southland (cf. Thompson (22)).

The species is also naturalized in waste places in the United States,

from Maryland and East Virginia to Florida, Arkansas and Texas; also in Bermuda.

The distribution of the species as a whole is thus consistent with the view that it is primarily a species of rather moist habitats, as indicated by



TEXT-FIG. 19. Map showing the distribution of *Ranunculus parviflorus* in southern England and France. Black regions indicate that the species is frequent or common; broken lines lower frequencies.

its anatomy, but that towards the limits of its geographical area it is found to be restricted to situations where the edaphic conditions determine an open community in which the growth of potential competitors is restricted.

The statement by Moss (12) that this species prefers calcareous soils is misleading. Actual cultures on non-calcareous loam, with and without added calcium, gave no support to this view, and indeed plants grown on

soils to which chalk was added were smaller than those grown on the same soil without chalk (Pl. XVIII, Fig. 9). The not infrequent presence of this species on calcareous soils is to be attributed to their physical attributes, which not only diminish the vigour of possible competitors but, by their comparative dryness and warmth in winter, ameliorate the rigours of a northern climate. The presence of *R. parviflorus* as a constituent in the ephemeral flora of sand dunes may be attributed to the same cause, and not to the comminuted shell fragments which such dune soils contain. That this species is not to be regarded as a calcicole is further shown by the great abundance which it attains on some of the arable fields of Devonshire, on soil derived from the non-calcareous old Red Sandstone.

The natural habitats in England are situations which tend to be arid in summer, often where the soil is very shallow, overlying rock. On these types of soil *R. parviflorus* can develop successfully with other species, since these competitors, even if potentially taller, cannot here attain to a sufficient stature to overshadow it, and the limited depth of soil tends to preclude robust perennials.

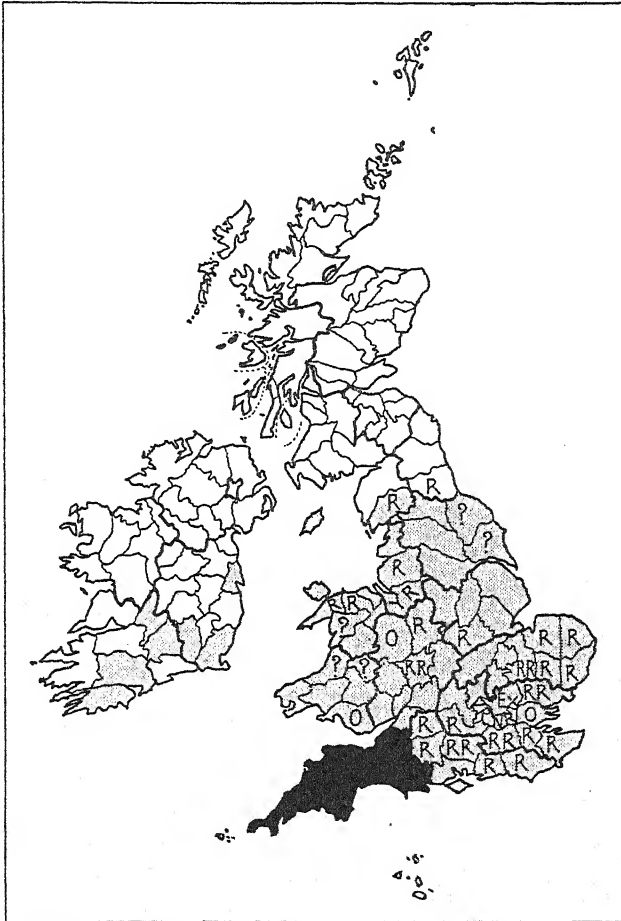
The species also occurs as a cornfield weed, where it attains much greater vigour and produces, as previously mentioned, many more fruits, attesting to the fact that the restriction to poor natural habitats is not because these are directly favourable, but because the edaphic conditions restrict the growth of *R. parviflorus* relatively less than that of its competitors. In the cornfield *R. parviflorus* is able to survive because the artificial conditions of culture maintain an 'open' plant community, and thus, too, preclude undue pressure from competition.

The common associates on shallow soils are such species as *Alchemilla arvensis* (the erect form), *Myosotis collina*, *M. versicolor*, *Montia chondrosperma*, *Valerianella olitoria*, *Erophila verna*, and *Saxifraga tridactylites*. All agree in being winter annuals like *R. parviflorus*, the brief life-history being mainly coincident with the period of maximum precipitation and humidity. Thus, although the natural habitats are dry, often extremely so at the time of fruiting, they tend to be moist in winter, especially having regard to the atlantic distribution.

As we pass to the warmer areas of its range, the species tends more and more to be restricted to moister situations, which suggests that the atlantic distribution is partly conditioned by rainfall, although the ameliorated minimal temperatures in an oceanic climate are perhaps even more important in consequence of the vulnerability of the species from severe frost resulting from its simultaneous germination.

The higher moisture demands towards the southern limit is shown by the occurrence in the Balearic Isles where, according to Knocke (10, p. 126), *R. parviflorus* is one of the species which, by their preference for the deep valleys of the Cordillère, betray their adaptation to the climate,

and in part their origin from central Europe. This inference of Knocke's is, however, hardly consistent with the distribution of the species, and especially its complete absence from central Europe.



TEXT-FIG. 20. Distribution of *R. parviflorus* in Britain. Frequent to common shown in black. O. occasional; R.R. rather rare; R. rare; V.R. very rare; EX. extinct.

In Cyprus, again, according to Holmboe, *R. parviflorus* is a plant of moist places (9, p. 82).

The detailed distribution in the British Isles is shown on the accompanying map (Text-fig. 20), in which the rarity in the east and north is noteworthy. In Ireland the area occupied is very limited, and Praeger refers to *R. parviflorus* as 'perhaps native, certainly naturalized in the South East' (Praeger (14)).

In England *R. parviflorus* has apparently become extinct in Hertfordshire from its former localities, and has decreased remarkably in

Oxfordshire. At the present day, except in the south-west, the plant is rare or very rare, yet we find that John Hill, in 'The British Herbal' published in 1756 (8, p. 16), under the name *R. hirsutus annus flore minimo*, states that our species was common on dry banks. Taken together, these and other records suggest that the species has decreased in frequency in England. Since there is evidence from biological and on other grounds that the conditions in England are in general drier, and that it is the aquatic and damp habitat species which have mainly decreased (cf. Salisbury, 19), the diminution of *R. parviflorus* must be attributed to special rather than to general causes. As a cornfield weed it has probably become less frequent, except in the West, since, like other southern species which were formerly introduced repeatedly with foreign agricultural seeds, modern screening methods have almost eliminated this means of introduction. But the diminution or extinction in more natural habitats is more probably due to the incidence of exceptionally severe winters. It is interesting to note its occurrence in the interglacial beds at West Wittering, Sussex (Reid (15)), since its climatic demands furnish further evidence of the mild climate of the interglacial period.

SUMMARY.

R. parviflorus is shown to exhibit a leaf arrangement consistent with the hypothesis that the apical meristem is the multicellular equivalent of a three-sided apical cell. The actual angular divergences between successive leaves range from 95° to 159° , with a mode in the neighbourhood of 120° . The only common feature is that there are three members in each completed turn of the spiral. It is suggested that this, too, is the only real significance of the Fibonacci series.

The structure of the leaf is described. It is shown to be stipulate and to be mesophytic rather than xerophytic in anatomy. The epidermal cells are remarkably irregular in outline, even in 'sun-leaves', and suggest a species adapted to moist rather than dry conditions. The observed stomatal frequencies range from 5 to 93 per sq. mm., the number of epidermal cells from 127 to 397, and the stomatal index has an average value of about 16. The aggregate stomatal aperture is about 1-3 per cent. of the total leaf surface. The irregular orientation of the stomata is shown to be present at their formation, and is not an outcome of the increasing irregularity of the epidermal cells as they mature. The stomata are raised above the level of the epidermis. Half stomata and twin stomata are described.

The ground tissue of the petiole and the cortex of the tetrarch root exhibit numerous and conspicuous intercellular spaces. The anatomy is thus in harmony with the atlantic distribution of the species.

Sections of very young flower buds show that the parts are produced in a sequence of three members in each completed turn of the spiral, those in successive turns alternating with one another, and here, as in the vegetative shoot, displacements can be attributed to mechanical pressure between the adjacent rudiments. The quincuncial calyx is held to represent two turns of three members each, in which one outer member and one inner member are congenitally fused. Hence in a trimerously spiral flower the total number of parts should be one less than a multiple of three (assuming a tendency for completed turns to be formed). Of 725 flowers examined in their entirety, the most frequent condition was 26 total parts (222 examples).

The structure of the sepals and transitional types indicate that they are derived from leaves and represent leaf-bases, thus being comparable to the protective bud-scales of some vegetative buds. Both leaf-base and sepal exhibit epinastic curvature and similar physiological response.

The occurrence of transitional types between stamens and petals, the spatial relations between them, and the fact that they show a negative correlation of $0.36 = 0.0218$, clearly indicate the staminal origin of the petals. The petals attain maturity after the stamens, which may be associated with their phylogenetically more recent origin. The petals bear nectaries which act as osmotic hydathodes, and bring about dehiscence of the stamens even in nearly saturated air.

Altogether 116 different types of floral organization were encountered, with the following ranges in the number of parts: K. 4-5, C. 0-5, A. 1-8, G. 3-18. The meristic variation is described, and the correlations between the different whorls determined.

The seed output per plant is shown to depend mainly on nutrition, and may be as high as 6,700, but does not normally exceed about 250 achenes. The percentage germination is high and may attain 100 per cent. Dispersal is probably largely by rain-wash.

The species is shown to be a 'winter annual'. Germination takes place in September, and is 'simultaneous'. In general, the achenes which do not germinate at this period are non-viable. Achenes buried artificially, or by earthworms, may be an exception, but in consequence of the normal simultaneous germination, and the susceptibility of the species to frost, it may be exterminated in a locality by severe weather.

Cultures have shown that the species does not grow so well on calcareous as on non-calcareous soils, but the association of *R. parviflorus* with shallow soils and dry habitats in Britain is conditioned by greater frost-resistance on these soils, and especially relief from the competition of tall species.

The distribution of the species is described and shown to be markedly atlantic in type. Its absence from central Europe is probably correlated

with the liability to severe frosts in a continental climate. In the warmest parts of its range the species is associated with damp habitats, which is in harmony with its structure and biology.

The histology exhibits several interesting and peculiar features. Both unicellular and multicellular archesporia are here recorded for the ovule, and also the occurrence of a tapetal cell. The ovule is anatropous before fertilization, but subsequently becomes amphitropous. The anatomy of the carpel shows an unreduced vascular supply of five traces, and the ovular trace may arise from one of the ventral strands, thus approaching the condition which obtains in a follicle. A swelling is met with above the single ovule in a position which suggests comparison with the second abortive ovule in some species of *Anemone*. The evidence of trimery which the species shows is thus in conformity with its retention of other primitive characters.

Postscript. It has been shown that the sepals are to be regarded as the morphological equivalents of leaf-bases. Under conditions of diminished illumination, as when *Ranunculus parviflorus* is growing where shaded by other vegetation, the leaves constituting the rosette become ascendant instead of adpressed, and the rosette habit is lost. This also occurs under like circumstances with *R. bulbosus*, *R. repens*, &c. which shows that a considerable intensity of light is necessary to induce the epinastic curvature of the leaf-base.

If, as is suggested, the reflexion of the sepals is a physiological response comparable to the photonastic reflexion of the leaves, it might be expected that the behaviour of the sepals would also be affected by the intensity of illumination. To test this, plants were grown in varying intensities of daylight, and it was found that when grown in diffuse light of 25 per cent. of that in the open, the sepals become normally reflexed, although the leaves of the rosette were ascending. When, however, plants were grown in a diffuse light of only 10 per cent., all the flowers produced failed to show reflexion of the sepals which remained erect throughout the life of the flower. In other respects the flowers produced were quite normal. These experiments thus demonstrate that the physiological behaviour of the leaf-base and the sepal is similar, although the light intensity necessary to bring about the epinastic curvature of the sepal is appreciably less than that requisite to bring about response of the leaf-base.

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EXPLANATION OF PLATE XVIII.

Illustrating Professor Salisbury's paper on *Ranunculus parviflorus*.

Fig. 1. Longitudinal section through ovule of *R. parviflorus* after fertilization, showing amphitropous stage. × ca. 15.

Fig. 2. Mature carpel prior to fertilization, in median longitudinal section showing the ovular trace and the median dorsal carpellary trace. The arrow points to the putative abortive ovule. × ca. 60.

Fig. 3. Young ovule in longitudinal section showing the two parietal cells beneath the epidermis, and division of the primary sporogenous cell into two. The single integument is beginning to develop, especially on the upper side. × ca. 380.

Fig. 4. Longitudinal section through a part of the flower showing an ovule in the pre-fertilization stage which is typically anatropous. The slight thickening of the carpel wall just above the funicle may indicate the rudiment of an abortive ovule. To the left a petal in longitudinal section shows the vascular strand expanding beneath the secretory tissue of the nectary. × ca. 90.

Fig. 5. Transverse section through a carpel showing the ovular trace arising from one of the two carpellary ventral traces (indicated by the arrow). × ca. 60.

Fig. 6. Longitudinal section of young ovule showing a multicellular archesporium. × ca. 360.

Fig. 7. Transverse section of a petiole showing the numerous intercellular spaces. × ca. 30.

Fig. 8. Transverse section of a very young flower-bud showing the trimerous arrangement of the parts. The three lobes of the central axis indicate the insertion of the three uppermost carpels, with these alternate three lower carpels sectioned just above the swollen region, and with these

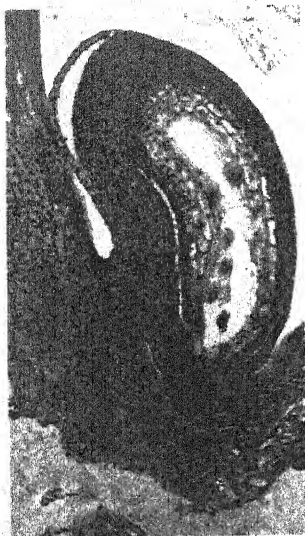
alternate three more carpels at a still lower level sectioned just below the stigmas (crescent-shaped). The next turn of the spiral consists of three stamens, and the next of two stamens and one petal. $\times 360$.

Fig. 9. Equal-aged cultures grown on sandy loam (left), and in the same soil with added chalk (right). $\times \frac{1}{2}$.

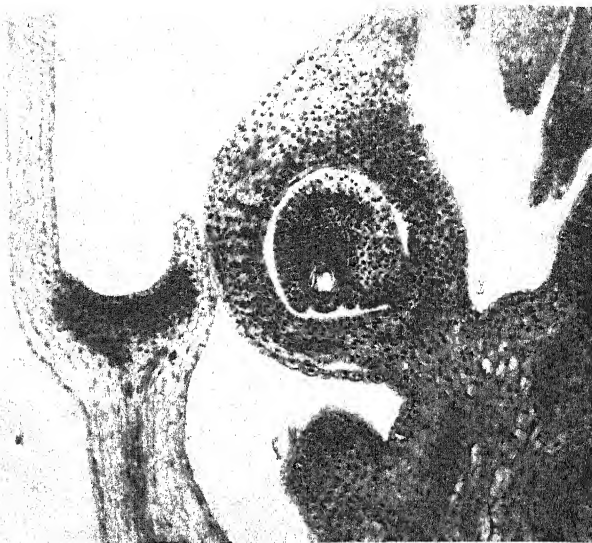
Fig. 10. Seedlings showing simultaneous germination. $\times \frac{1}{2}$.

Fig. 11. Epidermis of very young leaf showing the oblique orientation of some stomata, and indications of the subsequent sinuities of the vertical epidermal walls. $\times 360$.

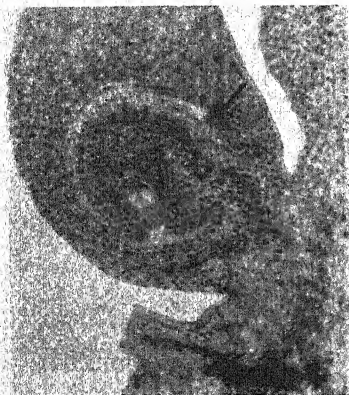
Fig. 12. Longitudinal section through apex of a plant in early March showing the terminal position of the flowers, and early maturation of the stamens. $\times 15$.



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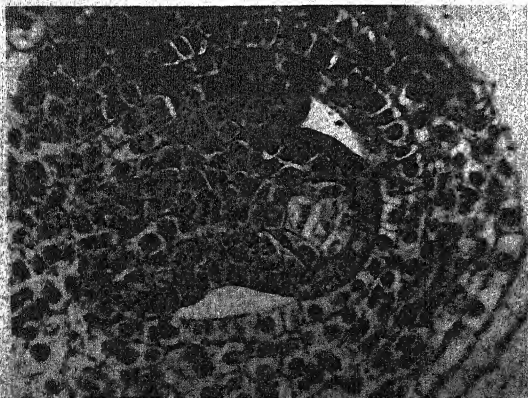
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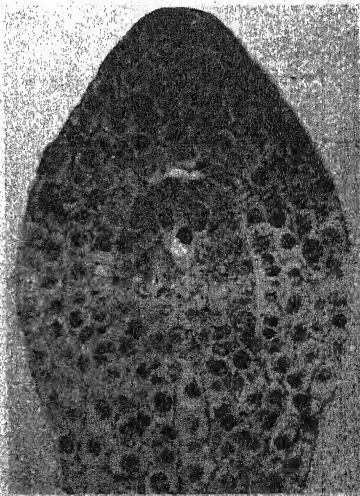
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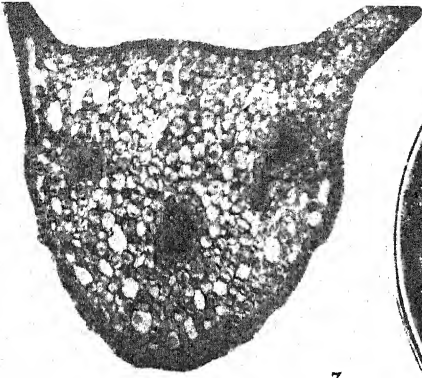


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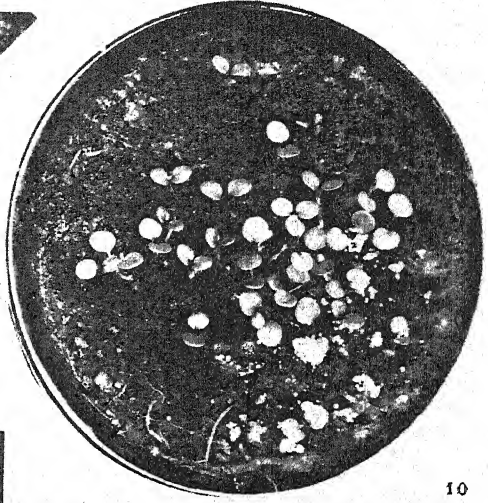


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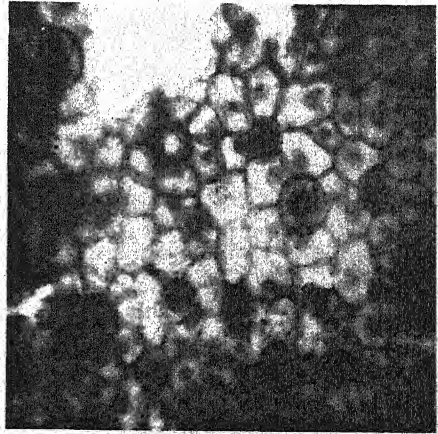
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A Physiological Study of Varietal Differences in Plants.

II. Further Evidence for the Differential Response in Yield of Barley Varieties to Manurial Deficiencies.

BY

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With one Figure in the Text.

IN a previous paper (3) the results of an experiment carried out in 1927 with five varieties of Barley were described. Evidence was there produced which indicates, with a very high degree of probability, that the comparative yields of varieties of the same species differ with the type of manuring given. This effect we have called the *differential manurial response*.

In the experiment of 1927 referred to, each of the principal manurial constituents—nitrogen, phosphorus, and potassium—were given in three stages of decreasing concentration, constituting nine 'deficiency series'. In each 'deficiency series' the two manurial constituents not in deficiency were supplied in the same concentration as in the control series with complete manure, while the third constituent was given in the ratio of 0.0, 0.04, and 0.2 of the complete amounts.

In view of the positive result obtained in 1927 it was decided to repeat the experiment, and this paper describes the result of the work carried out in 1928, again with Barley varieties. The following changes were introduced in this experiment:

(1) Of the five varieties previously used, only three were retained, of which two were the parent forms of the hybrid third. From the study of these three, it was thought that knowledge might be gained as to the inheritance of efficiency in utilization of mineral salts by Barley varieties.

(2) The large range of manuring used in 1927 resulted in very large variations in yield, and in the complete starvation series very small plants were obtained. Criticism might be levelled against these results on the ground that such completely starved plants are by no means typical, and it was thought it would be more profitable to study only the effect of moderate deficiencies. The number of manurial types was therefore reduced to four: three 'deficiency series' and a fully manured control.

(3) The results of 1927, previously published, referred only to the yields at time of harvest, and only one small sample was taken at the time of maximum vegetative growth. By reduction of the number of varieties and manurial types a sufficient number of replicates was available to allow of sampling at regular fortnightly intervals, throughout the growth period. In this way the differential response at different stages was followed from germination to harvest.

EXPERIMENTAL METHOD.

Three pure line varieties were used—Plumage (P), English Archer (E. A.), and the hybrid Plumage Archer (P. A.).¹

The experiment involved 504 pots, and was carried out in the open. The experimental procedure was similar to that described in the previous paper. The pots were of white glazed earthenware 10 in. by 10 in., holding 30 lb. of dry sand. Before use the sand was washed as carefully as possible by means of a rapid stream of water while being continually turned in a concrete lined tank. As more than 7 tons of sand had to be dealt with, it was impossible to use acid treatment for purification of the sand.

The complete manuring was reduced by a third from that used in 1927, but nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5) were again used in the ratio 3:2:1.

The 'deficiency series' contained the nutrient in minimum at a concentration of $\frac{1}{16.7}$ that of the control (exactly the same quantities as those of the sets B, F, J, in 1927), the other constituents being supplied at the same rate as in the fully manured set.

The scheme of manuring is given in Table I. The figures represent grammes per pot.

TABLE I.

	W. (Complete).	X. (Phosphate deficient).	Y. (Nitrogen deficient).	Z. (Potash deficient).
P_2O_5	0.333	0.020	0.333	0.333
N	1.0	1.0	0.06	1.0
K_2O	0.667	0.667	0.667	0.04

We have again to thank Dr. E. S. Beaven of Warminster for providing the seed.

The manures were added in solution as previously described, and brought to an initial pH 6.8 by use of sulphuric acid. Calcium (0.37 gm. CaCl_2), magnesium ($1.25 \text{ gm. MgSO}_4 \cdot 0.7 \text{ H}_2\text{O}$) and a trace of iron were added to each pot.

The seed was graded for uniformity in size and colour by eye, and nine seeds were sown per pot. The seeds were sown on April 20, uniformly at a depth of $1\frac{1}{4}$ in. below the surface, and germination began on May 2, 1928. In two days 94.7 per cent. of the seeds had germinated. The reduction in number of plants to three per pot was carried out as previously described.

Tiller numbers were counted each week, and leaf measurements were made on all series fortnightly, until the maximum leaf area had been reached.

From May 29 onwards fortnightly, until July 24, a random sample of generally six pots was taken from each series, and planimeter measurements of leaf area, together with dry weight data for green leaves, dead leaves, stems, and roots were obtained.

Harvest extended from August 23 to September 11, and each set in turn was harvested when ripe irrespective of the others. Ten pots remained in each series, except for the potash deficient, in which there were twelve.

A certain number of pots had to be discarded owing to injury from *Helminthosporium* in spite of the preliminary precaution of sterilizing the seeds with formalin, and some pots of the fully manured series had to be discarded owing to an obscure disorder leading to mottling of the leaves.

ANALYSIS OF DATA.

Table II presents the data of total dry weight of plants per pot at the various times of sampling indicated. The figures are the arithmetic means of the various sample weights. Differences in weight between the varieties occur in all the samples. A simple method of gauging the regularity shown by these varietal differences is as follows. After each entry in the Table a number 1, 2, or 3 is inserted showing the order of yield of the varieties within the particular manurial series at one time of sampling. Thus at the first sample of the fully manured series E. A. is marked (1), P. (2), and P. A. (3). By summing these values for the different samples within each manurial type, it is clear that if any one variety always occupied the first place the total would be six, whereas if one variety occupied consistently the last place the maximum sum would be eighteen. The results of the summation are seen in Table III.

From the figures in the Table the contrasting behaviour of Plumage and English Archer stands out very clearly; whereas with full manure and

TABLE II.
Total Dry Weight of Plants.
(Grammes per pot.)

	Date.	Sample No.	P.	E. A.	P. A.
W. Full manure	May 29	I.	1.51 (2)	1.61 (1)	1.29 (3)
	June 12	II.	8.71 (1)	6.87 (3)	8.18 (2)
	June 26	III.	32.41 (1)	24.39 (3)	29.39 (2)
	July 10	IV.	61.24 (2)	57.01 (3)	65.51 (1)
	July 24	V.	97.24 (1)	85.95 (3)	90.25 (2)
	Aug. 23	VI.	113.48 (1)	—	—
	Aug. 30	VI.	—	99.69 (3)	—
	Aug. 29	VI.	—	—	106.06 (2)
X. P ₂ O ₅ deficient	May 29	I.	1.02 (2)	1.05 (1)	1.01 (3)
	June 12	II.	3.62 (3)	4.53 (1)	4.08 (2)
	June 26	III.	7.84 (3)	8.76 (2)	9.81 (1)
	July 10	IV.	13.21 (2)	14.46 (1)	13.12 (3)
	July 24	V.	16.24 (3)	20.46 (1)	16.64 (2)
	Sept. 10	VI.	19.61 (2)	—	—
	Sept. 11	VI.	—	21.62 (1)	—
	Sept. 10	VI.	—	—	19.41 (2)
Y. N deficient	May 29	I.	1.29 (2)	1.41 (1)	1.21 (3)
	June 12	II.	6.15 (1)	5.79 (2)	5.52 (3)
	June 26	III.	8.93 (3)	10.63 (1)	9.34 (2)
	July 10	IV.	11.62 (3)	13.51 (1)	12.35 (2)
	July 24	V.	15.57 (2)	16.58 (1)	15.30 (3)
	Sept. 7	VI.	15.33 (3)	—	—
	Sept. 7	VI.	—	19.89 (1)	—
	Sept. 3	VI.	—	—	18.99 (2)
Z. K ₂ O deficient	May 29	I.	0.81 (1)	0.59 (3)	0.68 (2)
	June 12	II.	4.83 (1)	3.12 (3)	4.11 (2)
	June 26	III.	11.77 (2)	8.17 (3)	14.13 (1)
	July 10	IV.	18.57 (1)	12.30 (3)	17.65 (2)
	July 24	V.	26.56 (2)	23.93 (3)	29.62 (1)
	Sept. 3	VI.	20.47 (3)	—	—
	Sept. 4	VI.	—	21.51 (2)	—
	Sept. 6	VI.	—	—	23.84 (1)

TABLE III.

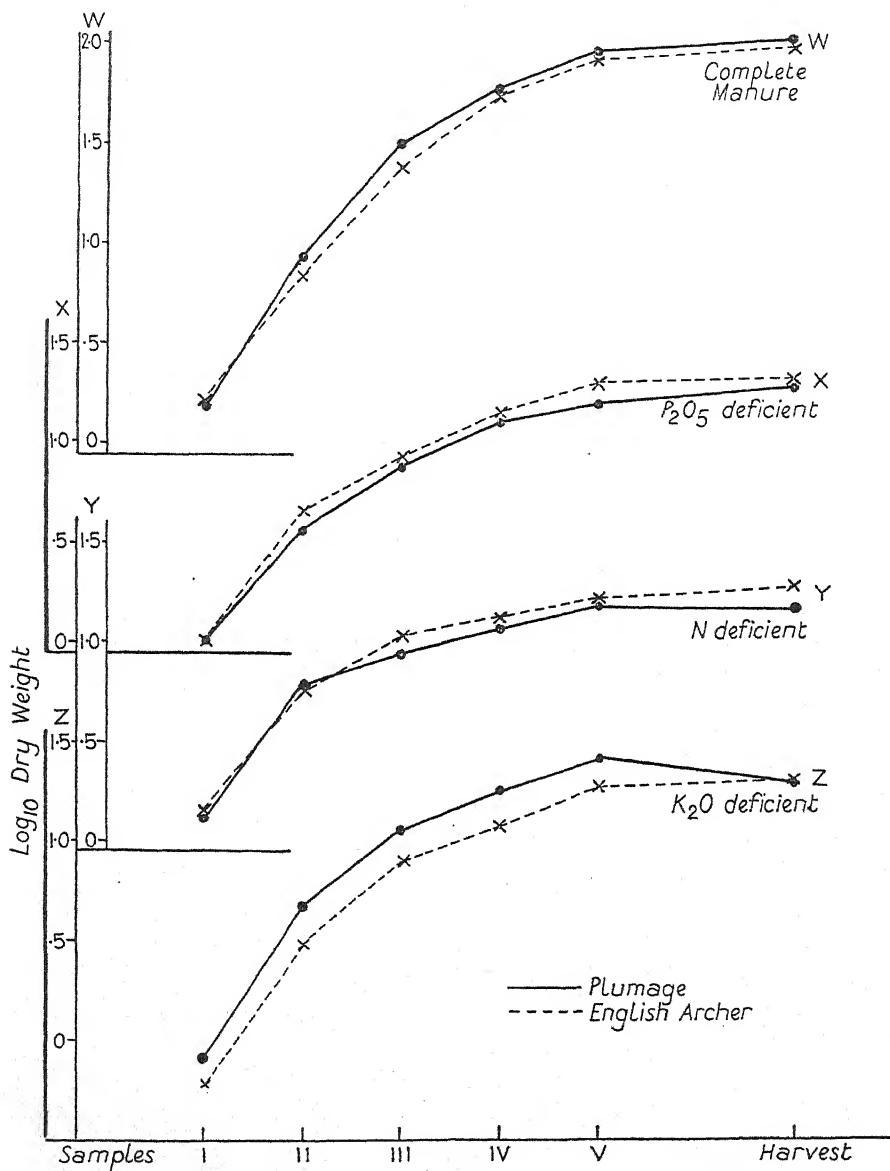
Manurial Type.	P.	E. A.	P. A.
W. Full manure	8	16	12
X. P ₂ O ₅ deficient	15	7	14
Y. N deficient	14	7	15
Z. K ₂ O deficient	10	17	9

in potash deficiency Plumage approaches the minimum value (6), English Archer under these conditions approaches the maximum (18). With nitrogen and phosphate deficiencies the reverse holds. In general Plumage Archer resembles one of the parent forms.

The dry weight figures are shown in graphical form on p. 583.

The values graphed are the logarithms of the dry weights for the

varieties, P. and E. A. in the four manurial types. The logarithms have been used instead of the actual weights to reduce the discrepancy in the



Relative dry weight increase of varieties Plumage and English Archer with complete manuring and three deficiencies.

scale of the various graphs. It is seen that the curves only rarely cross, and the varietal differences tend on the other hand to be cumulative, i.e. on the logarithmic scale the curves run almost parallel.

The foregoing considerations indicate strongly that a real effect is being studied, but to carry conviction a thorough statistical examination is essential. The analysis of variance has been utilized for this purpose. The variables concerned in the analysis are as follows :

Three varieties (P, E. A., P. A.) with two degrees of freedom ; four treatments (fully manured, N, P, K deficiencies) with three degrees of freedom ; six harvests giving five degrees of freedom, and finally six replicates of each combination of treatment and variety. The total possible number of individual entries is thus $3 \times 4 \times 6 \times 6 = 432$, with 431 degrees of freedom. The analysis of variance may thus be written :

	Degrees of freedom.
Varieties	2
Treatments	3
Time	5
Remainder	
Error	360
Interactions	61
Total	431

The variety and treatment variances are obtained by grouping all the experimental results first under the headings of the three varieties, irrespective of treatment and sample number, and summing the values ; then grouping under the heading of the four treatments, irrespective of sample number and variety, and again summing the values. From the three totals of the data grouped under variety, and the four totals grouped under treatment the sums of squares of differences from the means of the totals are calculated, and reduced to the basis of a single pot yield by dividing by the number of individual yields summed in the total.

The time variance represents the contribution to total variance made by the variation in weight of the several harvests. It is estimated by summing all the yields at the time of each harvest, thus obtaining six totals from which the variance is calculated. The combinations of treatment and variety are each replicated six times, each group of replicates giving 5 degrees of freedom for estimation of errors. Since there are in all $4 \times 3 \times 6$ groups of replicates, the total number of degrees of freedom for estimation of error is thus $4 \times 3 \times 6 \times 5 = 360$.

The 61 degrees of freedom representing the interactions of the variables may be further subdivided as follows :

Interactions.	Degrees of freedom.
<i>Variety v. Treatment</i>	6
Variety v. Time	10
Treatment v. Time	15
Variety v. Treatment v. Time	30
Total	61

Each of these interactions gives important information as to the behaviour of the plants under treatment.

First: the interaction of variety and treatment measures the variations in the comparative yields of the varieties studied under the different manurial treatments, and is the Differential Response to Manuring which the experiment was designed to investigate.

Secondly: the interaction of variety and time measures the variations in the comparative yields of the varieties at the different harvests, and thus shows whether the time relation of the relative growth rate differs between the varieties.

Thirdly: the interaction of treatment and time measures the variations in the comparative yields of the plants (irrespective of variety) at different sampling times; in other words, it measures the variations between the relative growth rates due to different manurial treatments.

Lastly: the second order interaction between variety, treatment, and time differs from the previous interaction of treatment and time, in that the differences between the varieties in this respect are taken into account; in other words, it measures possible variations in the differential response of the varieties at different stages in the life-history.

The method of calculating these interactions cannot here be described in detail, and reference should be made to Fisher's book (2).

TABLE IV.

		Manurial treatments.				
		W.	X.	Y.	Z.	
Varieties	P.	S(x)	116·36226	69·76842	72·62067	78·45162
		x	3·232285	1·938012	2·107241	2·179211
		xS(x)	376·115988	135·212035	146·493393	170·962633
	E. A.	S(x)	111·54395	74·65305	75·63682	68·15507
		x	3·098443	2·073696	2·101021	1·893196
		xS(x)	345·612571	154·807732	158·914547	129·030906
	P. A.	S(x)	113·78161	61·82502	73·23259	78·45760
		x	3·160600	1·995139	2·034239	2·179378
		xS(x)	359·618157	143·300899	148·972591	170·988767
	General sum		= 1004·48868			
	General mean		= 2·325206			
	General xS(x)		= 2335·642148			
Total xS(x)		= 2440·030220				
General xS(x)		= 2335·642148				
Total sum of sqq.		= 104·386672				
Sum of sqq. for varieties		= 0·244400				
		104·142272				
Sum of sqq. for treatment		= 101·605418				
Sum of sqq. for diff. response		= 2·536854				

Since the variety v. treatment interaction is of prime importance here, the calculation for the total dry weight is given above (Table IV).

The figures entered in the Table constitute twelve groups which are the combinations of varieties and treatment. Each group consists of three values, namely:

(a) $S(x)$ which is the summation of all the replicates for a particular combination of manuring and variety at each harvest, and thus is the sum of thirty-six individual values, i.e. the six replicates at each of the six harvests.

(b) \bar{x} , which is the mean value for the particular variety with a given treatment, and is obtained from the value $S(x)$ by dividing by the number of replicates, i.e. by thirty-six.

(c) $\bar{x}S(x)$ which is the product of the two previous values.

In addition the general sum and general mean and their product are given. These are obtained by summation of the twelve values of the $S(x)$, deriving the general mean by dividing by the total number of replicates (432), and then multiplying these together.

By adding the individual values of $\bar{x}S(x)$, and subtracting from their sum the general $\bar{x}S(x)$, the remainder gives the sum of the squares of deviations of the means in the Table from the general mean. This result is on the basis of a single pot yield. By subtracting successively the sum of squares for varieties and treatment, the final remainder gives the sum of squares for differential response on a single pot basis.

The other interactions are obtained in a similar manner.

Preliminary Treatment of the Data.

Before discussing the results of the analysis of variance, the following considerations must be noted:

(1) A preliminary examination of the dry weight data showed that the variance within each treatment was not the same, as was found also in 1927. Instead of the actual values of yield, the natural logarithms of the yields were therefore used, and now the relative variances were found to be nearly constant. A consequence of using the logarithms is that the means of treatments and varieties obtained by taking the means of the logarithms and then finding the antilogarithms are not the same as those in Table II, since the latter are arithmetic means, whereas the former are the geometric means. The differences are in general small.

(2) Owing to the necessary discarding of some of the fully manured plants, it was not possible at each harvest to sample the full complement of this treatment. In such cases use was made of a method of estimating the missing yields recently published by Allan and Wishart (1). In all cases where this was done, the requisite number of degrees of freedom were eliminated, and for this reason the total number of degrees of freedom

in the full analysis falls below the maximum possible number of 432, the difference indicating the number of estimated yields used.

(3) Separate records were made of total dry weight, green leaf, stem, root, and ear weights. A complete analysis of variance of each part was made.

TABLE V.

Values of 'Z' for Significance of Differential Response.

Sample No.	Green leaves.	Stems.	Roots.	Ears.	Total.
I.	0.3482	0.2987	0.1474	—	0.3180
II.	0.1991	0.0951	0.3580	—	0.3541
III.	0.8973	0.2795	0.3097	—	0.4119
IV.	0.8346	0.3413	0.8029	—	0.5218
V.	0.2181	0.1432	0.3486	—	0.3423
5 % probability value of Z 0.4064 { $n_1 = 6$ }					
1 % " " " 0.5687 { $n_2 = 60$ }					
Harvest					
		0.4769	0.1987	0.5352	
5 % probability value of Z 0.3905 { $n_1 = 6$ }					
1 % " " " 0.5449 { $n_2 = 108$ }					
	Green leaves.	Stems.	Roots.	Ears.	Total.
All samples combined	0.8586	0.7678	0.7752	0.5352	1.0565
5 % probability value of Z	0.3781	0.3769	0.3769	0.3905	0.3769
1 % probability value of Z	0.5256	0.5245	0.5245	0.5449	0.5245
	{ $n_1 = 6$ }	{ $n_1 = 6$ }	{ $n_1 = 6$ }	{ $n_1 = 6$ }	{ $n_1 = 6$ }
	{ $n_2 = 284$ }	{ $n_2 = 344$ }	{ $n_2 = 344$ }	{ $n_2 = 108$ }	{ $n_2 = 344$ }

The results of the analysis are shown in Tables V and VI. To economise space all the analyses are not given in full, and those in Table VI are only the complete analyses for total dry weight and for the several parts of the plants, utilizing the complete data from all samples.

The significance of the results is assessed by the 'Z' test (Fisher), in which the variance due to any known cause is compared with the variance due to error.

The values of 'Z' for the differential response are given in Table V, which tabulates the results of the analyses on the separate samples for the different parts of the plants. In the Table are given the 5 per cent. and 1 per cent. probability values of 'Z', which are the values which 'Z' must attain to assure a probability of 20 to 1 and 100 to 1 respectively against the result obtained being merely due to chance. The significant results in the Table are shown in heavy type.

The results of the analysis of the single samples show that the

TABLE VI.

Complete Analysis of Variance for Total Dry Weight and Weights of the Several Parts.

Green leaves.

	Degrees of freedom.	Sum of squares.	Mean square.	'Z'.
Time	4	204·511028	51·127757	
Variety	2	0·014416	0·007208	
Treatment	3	102·503568	34·501189	
Time v. Variety	8	2·823010	0·352876	
Time v. Treatment	12	52·973403	4·414450	
Variety v. Treatment	6	1·878819	0·313136	0·8586
Time v. Variety v. Treatment	24	1·598839	0·066618	
Error	284	15·968688	0·056228	
Total	343	418·271771		

Stems.

	Degrees of freedom	Sum of squares.	Mean square.	'Z'.
Time	5	995·973070	199·194614	
Variety	2	0·712820	0·356410	
Treatment	3	153·150291	51·050097	
Time v. Variety	10	1·675143	0·167514	
Time v. Treatment	15	35·370107	1·010575	
Variety v. Treatment	6	2·284749	0·380792	0·7678
Time v. Variety v. Treatment	30	1·827061	0·060902	
Error	344	28·234619	0·082078	
Total	415	1219·227860		

Roots.

	Degrees of freedom.	Sum of squares.	Mean square.	'Z'.
Time	5	398·178945	79·635789	
Variety	2	0·622929	0·311465	
Treatment	3	57·194624	19·064875	
Time v. Variety	10	1·166338	0·116634	
Time v. Treatment	15	22·886608	1·525774	
Variety v. Treatment	6	2·435408	0·405901	0·7752
Time v. Variety v. Treatment	30	4·307857	0·143595	
Error	344	29·628686	0·086130	
Total	415	516·421395		

Ears.

	Degrees of freedom.	Sum of squares.	Mean square.	'Z'.
Variety	2	4·109391	2·054696	
Treatment	3	174·535819	58·178606	
Variety v. Treatment	6	4·123178	0·687196	0·5352
Error	108	25·446395	0·235615	
Total	119	208·214783		

Total dry weight.

	Degrees of freedom.	Sum of squares.	Mean square.	'Z'.
Time	5	597.257639	119.451528	
Variety	2	0.244400	0.122200	
Treatment	3	101.605418	33.868473	
Time v. Variety	10	0.545251	0.054525	
Time v. Treatment	15	29.234439	1.948963	
Variety v. Treatment	6	2.536854	0.422809	1.0565
Time v. Variety v. Treatment	30	0.895209	0.029840	
Error	344	17.581729	0.051110	
Total	415	749.900939		

differential response increases with time: thus in the first two samples there are no significant results, but in the third and fourth samples the results for the total dry weight are significant, and also for the green leaves, and in one case the roots. In sample V there are again no significant results, but in the final sample both stems and ears show highly significant values. When all samples are combined every part of the plants, as well as the total, gives values with a significance greater than 100:1 probability, except the ears which have a value slightly below 100:1 probability. Statistically, therefore, the reality of the differential response is established with a very high degree of probability, and thus the experiment here detailed confirms the finding of the previous year.

Examination of Table VI shows the following effects of the single factors and interactions. The effects of time and treatment are in every case very great, as would be expected. As to the other factors the results are tabulated in Table VII below.

TABLE VII.

Factors.	Green leaves.	Stems.	Roots.
Variety	Not significant	Significant	Significant
Time v. Variety	Significant	Not significant	Not significant
Time v. Treatment	Significant	Significant	Significant

The Table shows that whereas the relations of leaf-growth rate to time vary in the different varieties, this is not true of the stems and roots. On the other hand, the leaf growth of the various varieties *on the average* does not differ significantly, whereas the stem and root growth do differ. In all cases the variations in relative growth rates due to manurial treatment are significant.

The evidence presented above establishes the fact of the differential response, and as three of the varieties were grown in the two consecutive years, it is possible to make a comparison between the behaviour of these varieties under deficiency in the experiments of 1927 and 1928. For purposes of comparison the values for the three stages of deficiency of

1927 are added together, and also the values of the various sample weights for each manurial series in 1928.

The results are presented in Table VIII as relative figures, the value for Plumage being always taken as 100.

TABLE VIII.

	1927.			1928.		
	P.	E. A.	P. A.	P.	E. A.	P. A.
P ₂ O ₅ deficiency	100	111	93	100	115 (110)	104 (99)
N deficiency	100	113	120	100	115 (130)	106 (124)
K deficiency	100	105	108	100	84 (105)	108 (116)
Full manure	100	97	97	100	88 (88)	96 (93)

Comparing the values in the Tables for the two years, it is seen that, so far as Plumage and English Archer are concerned, the relative yields are in the same direction in the two years, with the exception of the potash deficient series. It must be remembered, however, that the figures for 1927 refer only to the harvest weights, while those for 1928 are average values for the whole growing period. The figures in brackets for 1928 give the relative yields at harvest only, and the agreement is now much better. In two cases, namely, P and K deficiency, the order of yields for the three varieties is exactly the same in each year; in N deficiency the order of E. A. and P. A. are reversed, while in the fully manured series the values of E. A. and P. A. are identical in 1927, but not in 1928. As regards the three varieties studied in 1928 as well as 1927, the conclusions may be summarized thus:

Under conditions of high fertility Plumage gives a greater yield than English Archer, whereas under low nitrogen and phosphorus supply Archer gives a higher yield than Plumage. Under potash deficiency at harvest English Archer appears to give a higher yield, although throughout the vegetative phase Plumage consistently leads.

Plumage Archer appears to suffer more than either parent from phosphorus starvation, or at least closely resembles Plumage in its phosphorus requirements; under nitrogen starvation it resembles English Archer more closely; under potash deficiency it appears to be definitely superior to either parent, whereas with full manuring it is intermediate.

In the interpretation to be put on the results obtained in these experiments, the question of variations in quantity of reserves of the nutrients studied in the seeds of the varieties used must be considered.

Data are available on this point for the seed used in 1927, and are given in Table IX. The figures entered are the sum totals of dry weight of plants from each of the three stages of the deficient nutrients respectively, and the percentage of the particular nutrient in samples of the actual seed of the different varieties used in the experiment.

TABLE IX.

Variety.	P ₂ O ₅ deficient.	% in seed.	N deficient.	% in seed.	K deficient.	% in seed.
Goldthorpe	91.85	1.01	43.44	1.36	98.84	0.437
Plumage	96.91	1.08	51.13	1.65	78.95	0.578
English Archer	108.58	1.12	57.94	1.70	83.22	0.684
Plumage Archer	89.76	1.03	61.44	1.58	85.20	0.593
Spratt Archer	88.14	1.11	53.95	1.66	81.16	0.696

The figures show no clear relation between the final yield and the amount of reserve nutrient in the seed, and hence this factor may be assumed to have been unimportant in determining the results obtained in these experiments.

In conclusion it may be claimed that the results of these two years to a large extent corroborate each other, and establish with some certainty the differential manurial response. Further, it appears that the varieties Plumage and Archer differ widely in their efficiencies in the use of the mineral nutrients, and the widespread success of Plumage Archer may be attributed in some measure to the fact that this variety combines in itself the higher efficiencies of both its parents.

SUMMARY.

Further evidence is presented for the existence of a differential response of varieties of barley to various types of manuring.

Three varieties were grown, namely, the hybrid Plumage Archer and the parent forms Plumage and English Archer. Four types of manuring were used, fully manured controls, and deficiency in nitrogen, in phosphorus, and in potassium. 42 replicates of each variety for each manuring were used, giving 504 cultures in all.

Each set of replicates was sampled fortnightly throughout the period of vegetative growth, each harvest representing a random sample of six pots.

The resulting data were treated by the analysis of variance method, and values for differential response of significance far greater than 100 to 1 are obtained for each part of the plant.

The behaviour of the varieties is compared with the previous results of 1927, and a large measure of agreement is found, showing that with some certainty the varieties studied may be characterized by their manurial efficiencies.

The hybrid form appears to inherit specific manurial efficiency from both parents.

In conclusion the authors wish to express their thanks to Sir John

Russell for facilities for carrying out the experiment at the Rothamsted Experimental Station; to Professor V. H. Blackman, for his continued interest; to the Statistical Department of Rothamsted for advice; and finally to Dr. E. S. Beaven for helpful criticism and encouragement.

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A Contribution to the Physiology and Anatomy of Tracheae, with Special Reference to Fruit Trees.

I. Influence of Tracheae and Leaves on the Water Conductivity.

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With eight Figures in the Text.

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INTRODUCTION.

THE mechanism of transpiration in higher plants has received considerable attention. In general, it is quite well agreed that the total loss of water from plants depends (a) on the rate of the movement of water in the soil, (b) absorbing power of the root, (c) conducting or

transporting power of the plant, and (d) the transpiring power of the leaves. Each one of these factors is influenced by either the external or internal conditions, or both, as stated by Palladin (26) and others.

The third factor stated above, namely, the conduction of water in higher plants, is very important, both from the standpoint of physiology and anatomy.

In angiosperms (with few exceptions) the tracheae are concerned in carrying water and mineral nutrients, being especially fitted for this purpose, as stated by Jeffery (22), Eames and MacDaniel (8), and Haberlandt (14). The tracheae are continuous tubes extending from the finest branches of the roots, up through the stem, and into and throughout the leaves as pictured by Stevens (34). It may be mentioned, as has been shown by Curtis (4) (5), that only water and inorganic substances are transported upward by tracheae and not the organic materials.

There is some data available on the water conductivity in relation to the anatomy of tracheae. In all these studies the correlation between water conductivity and number, size and length of tracheae, has been brought out. So far as the writer is concerned, however, the data on water conductivity is not convincing for several reasons, which will be pointed out later.

In this study an attempt has been made to find :—

- (a) The length and distribution of tracheae.
- (b) The number of tracheae at various levels of the shoot.
- (c) The area of the cross-section of tracheae per unit area of cross-section of wood.
- (d) The distribution of the tracheae in different annual rings.
- (e) The relationship between number and area of the leaves and cross-section area of the tracheae.
- (f) The significance of the last-formed wood in conductivity. It is hoped that this data may be of some help in elucidating some of the factors concerned in the mechanism of water conductivity.

HISTORICAL REVIEW AND COMMENTS.

In 1915 Dixon and Marshall (7) showed that in cross-sections of *Acer pseudoplatanus* wood, 28.2 per cent. of the area was lumina of parenchyma cells, 33.5 per cent. was lumina of tracheae, and 38.2 per cent. was area of the walls. In a similar manner they found that in *Cotoneaster frigida* the mean area of the lumina of parenchyma cells was 7.4 per cent. of the total cross-section area, the total area of the lumina of tracheae was 49.8 per cent. and area of the walls was 42.8 per cent. From their study they concluded that the area of the lumina of tracheae are variable for different woods, ranging from 33.5 per cent. to 57.4 per cent. of the cross-section area.

In the quantitative estimation of the elements of the wood, Dixon

and Marshall used a camera lucida drawing, or a micro-photograph of a transverse section of the wood. They then cut out the walls. The lumina of the vessels and the cells were kept apart and weighed. From these weights the percentages of the total cross-section occupied by the walls and the lumina of the various elements were deduced.

The writer repeated this method, and found 15 to 18 per cent. error, which is beyond the range allowable for experimental error. Some of the factors responsible for this error were experimentally found, namely:—

1. *The difference in the weight of papers. (Probably due to quality of fibre and pressure.)*

This difference was true of photographic or others of the same quality or brand. Twenty-five different brands of paper (three of which were obtained from England), including developing papers, were checked, and the mean error due to differences in weights of the same brand of paper was found to be 7 to 9 per cent. This conclusion is based on the data obtained from the triplicate weights of the same known unit area.

2. *Error due to weighing. (Area-weight.)*

Not only a difference due to the weight of a unit area of the same type of paper but also an error due to the area-weight relationship was found. This was found to be particularly true in weighing a paper occupied by a cross-section, magnified 600 times.

From twenty-five duplicate readings it was ascertained that values per unit area and weight were constant up to 4.2 square centimetres, beyond which this consistency could not be held. Between 8- to 10-square centimetres a 5 per cent. error was found (after deducting 7-9 per cent. as noted under 1).

3. *Error due to photographic papers after developing.*

Developing involves chemical reactions. A given surface of a photographic paper, when exposed to a beam of white light (as is done in printing from a developed negative) is allowed to react photochemically. The quantity of such a reaction is different on the various parts of the same surface, even under the same intensity of light, because the developed negative transmits different rays when exposed to different objects or different parts of the same object. Eventually such a photochemical reaction is followed by differences in ordinary chemical reactions, which take place in the process of printing. Thus it is not surprising that two unmounted photographs of different objects, even of the same objects in different positions, show differences in area-weight, as is shown below.

Thirty 8-square centimetre photographic papers were weighed very accurately under red light used by photographers. Six negatives from different locations of the same cross-section (two-year apple shoots) were obtained. From each negative five prints were made. The areas were measured again and the weights were recorded. Although areas varied

but very slightly, no single lot had the same weight as before. This difference between the maximum and the minimum was 10 per cent., while the average was 7.2 per cent. In this error of 7.2 per cent. the previous errors (7 to 9 + 5 per cent.) were not included.

Farmer (11) (12), by measuring the amount of water in a given time and at a standard pressure, passing through a definite length of twig or branch (the cross-sectional area of which was ascertained) found that evergreens, as a class, possessed wood of markedly lower water conducting efficiency than that of broad-leaved deciduous trees. This he found was due to the fact that evergreens had narrower and shorter vessels. This seems reasonable, since the short length of vessels involves greater resistance to the passage of water. Farmer's data indicated there was a falling off in the amount of water transmitted, owing to the narrowing of the diameter of the stem towards the apex. He also concluded that the specific conductivity was often lower at the base than higher up, where the lateral leaf-bearing shoots are chiefly produced. The absolute volume of transmitted water was greater at the base owing to its greater cross-sectional area. Farmer used statistical methods, and he recognized the fluctuations due to the lack of homogeneity in the nature of the material, especially in the case of deciduous trees. However, due to the lack of more accurate methods of analysis, the statistical one seems to be the most satisfactory way of interpreting the results.

Holmes (17) (18) made observations on the anatomy of ash wood and hazel wood with reference to the water conductivity. She recorded number, size, and distribution of the elements in the wood concerned. She found that specific conductivity in the ash wood was lower than in hazel wood, because the hazel wood had a greater number of conducting elements per mm. than the ash wood, although these less numerous vessels were wider on the whole. In other words, Holmes attributed the relatively lower specific conductivity of ash wood to its relative poverty in the number of vessels, which in spite of their large area did not compensate for the larger number of vessels present in the hazel wood. It may be pointed out that the number of elements may not always be correlated with the specific conductivity. The data presented in this paper seems to show that there is more correlation between specific conductivity and area of the tracheae than between the number of the tracheae and specific conductivity. However, it is quite possible, as Holmes has shown, that in some cases a greater number of elements may compensate for the larger area.

Holmes's papers quoted above have to be considered critically from two standpoints.

A. She measured the diameter of the cavity of every water-conducting element by means of an eye-piece micrometer. From this diameter she

has computed the areas of these elements. She herself admitted that all vessels were not circular, consequently the results must be accepted comparatively only. Experience in measuring areas of vessels on millimetre-squared paper, and computing from the diameter with the assumption that tracheae are circular, has shown that, whereas there is 2 to 3 per cent. experimental error in the former method, there is 10 to 13 per cent. error in the latter.

B. In all her studies she used one-year-old shoots. Farmer (11) (12) has shown that toward the base there is an increase in mechanical tissue and decrease in tracheae per unit area of wood. It seems probable that in hazel and ash wood there may be an uneven increase of mechanical tissue or an uneven decrease of tracheae toward the base. Therefore, such a relationship of water conductivity, with number or area of tracheae as shown by Holmes, may not hold true at those levels of the shoots which are more than one year old. In fact apple and prune shoots do not behave similarly when shoots of three years are substituted for those of one year, even though such shoots are taken from the same tree. Data to that effect is presented elsewhere.

Rivett (28) worked on the anatomy of *Rhododendron ponticum* L. and *Ilex aquifolium* L. with reference to the specific conductivity. By forcing India ink through the wood under pressure, she found that the tracheal length of different shoots of *Rhododendron ponticum* varied from 6 cm. to 16 cm. From these data she concluded that it was the length of the tracheae rather than the balance between the size and the number of the tracheae which was the important factor in conductivity. All the contributions mentioned above (although they do not recognize the importance of number, size, and length of the tracheae with reference to water conductivity in the same way) agree that there is a general decrease in absolute conductivity from the base upwards. At the same time there is an increase in the specific conductivity from the base of a shoot toward its apex.

Inamdar and Shrivastava (20) have studied the seasonal variation in specific conductivity of wood in tropical plants with reference to the leaf fall. They maintain that the supply of water to leaves depends not merely on the external supply to the roots, but also on the capacity of the conducting tissues to carry water. Their data show a correlation between the period of leaf fall and the increased capacity of the wood for conducting water.

Inamdar and Shrivastava's (21) experiments, like those of Kohl and Schenck's on a number of plants, indicated that the development of vascular systems was necessarily correlated with the physiological necessities of the plants for water supply. These results are very interesting because they show that there is a variation in the conducting capacity of the tracheae at different times of the year. It may be that in the physiological

auto-regulation viscosity of the liquid passing upward varies at different times of the year. Since Inamdar (19), did not consider the mechanism of physiological auto-regulation, it is difficult to decide the matter from his data, although there seems to be a strong probability that toward leaf-fall sap extracted from tracheae may be more viscous. Even in plants of temperate regions, viscosity is highest during those periods when water conductivity is least.

For some time now phytopathologists have been injecting foreign substances for the control of sap-sucking and wood-boring insects by poisoning the sap of trees as indicated by the research of Rankin (27), Sanford (31), Shattuck (32), Moore, William, and Ruggles (25), and Flint (13). Such studies have brought forth information concerning the path of conduction. For instance, Elliot (9) injected potassium cyanide into apple and willow trees, either under the bark or in the wood of the current year's growth. His experiment showed that the injury never extended to the rings of growth, which were not in contact with the cyanide. Where the potassium cyanide was successfully kept out of the current year's growth there was no external appearance of injury to the branches. Still stronger evidence that water was conducted in the current year's wood seems to have been brought forward by Rumbold (29). She injected solutions of eosin, methyl green, and congo red into chestnut trees, and found that in no case did they spread beyond the beginning of the new year's growth of twigs and roots. Most of the dye passed through the xylem element of the last-formed annual ring. In the injection method it is never possible to cut a hole so that air can be excluded, as pointed out by Rumbold (30). The passing of air into the tracheae involves another difficulty in studying the ascent of sap, as discussed by Ewart (10) and Hartig (15).

Furthermore, as a hole cannot be cut cleanly, tracheae may be partially plugged by the small particles of the cut wood. As the result of such a plugging, the conduction would be slow; thus the diffusion from the tracheae to the surrounding cells would be greater, as Elliott (9) seems to have shown by his work. As a result of slower conduction (due to several of the above factors) dye might pass to the adjoining wood from the wood in which actual conduction took place, thus it could appear that all the wood took part in water conductivity. There is some evidence on record to substantiate this statement.

MATERIAL AND METHODS.

A. The length, number, and distribution of tracheae in relation to water conductivity.

About a dozen uniform shoots of various lengths, ranging from 15 cm. to 100 cm., and of different ages varying from one year to three years, were

obtained from trees with pome, drupe, nut, and citrus fruits; several varieties of grapes were also used. All the shoots used for this study were obtained from the horticultural plot of the University of California (Berkeley), and were grown under very similar environmental and edaphic conditions. The leaves were immediately stripped off, and the shoots were wrapped with cheese cloths which were kept moist to avoid

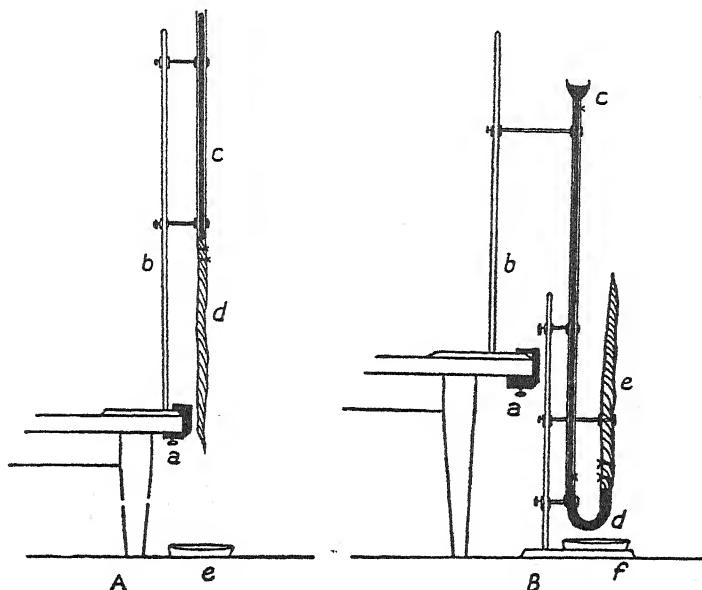


FIG. 1. Device used for forcing mercury. A. Device for smaller shoots. (a) Clamp to hold the apparatus. (b) Iron rod fitted with two clamps to hold the tube in place. (c) Heavy glass tube with mercury. (d) Twig (covered with wet cheese cloth) connected with the tube by means of a strong rubber tubing fastened tightly by means of steel wire. (e) Pan to catch the falling mercury. B. Device for larger shoots. (a) Clamp. (b) Iron rod fitted with a clamp. (c) Reservoir for mercury. (d) Strong rubber tubing supported by clamps. (e) Shoot (base below, top above). (f) Pan for falling mercury.

drying of the shoots. Mercury was forced into the base of the xylem under a pressure of 60 cm. in case of the smaller shoots and under a pressure of 100 cm. in case of the larger ones, thus avoiding the possibility of error due to plugging of vessels with air. The devices used for forcing mercury through both kinds of shoots are shown in Fig. 1. After treatment for thirty-four hours, lengths of the shoot from 0.2 cm. to 0.5 cm. were cut off, beginning at the tip, and the number of points at which mercury appeared on the cut surface was observed by a hand lens. It was assumed that mercury passed into all the vessels until a cross wall was reached. When it was impossible to count with a lens the large number of individual points at which mercury appeared the remainder of the shoot was cut into a series of lengths of about 1 cm. from which sections were prepared for examination under the microscope.

The number of spots of mercury indicated the number of vessels open from the base of the shoot up to the portion of the section under observation. At each cut the length of the remainder of the shoot, as well as the diameter at the point of the cut, was recorded.

Since the density of mercury is very much higher than the density of water or of air it was possible that the mercury might not reach the same height as water or air. Therefore the length of the tracheae

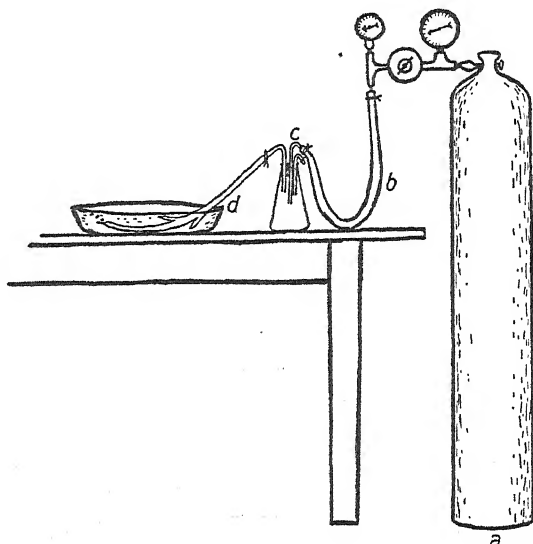


FIG. 2. Device used for forcing gas (nitrogen) through shoots. (a) Gas cylinder fitted with pressure meters. (b) Heavy rubber tubing. (c) Heavy pyrex flask with tube for gas outlet, thus reducing pressure. (d) Shoot with the base end fitted in the rubber tubing and the apex submerged in water. Bubbles are arising from the ends of tracheae.

was also determined by passing gas (nitrogen), under a pressure of one atmosphere, through branches from the base, submerging the tip in water. The shoots were cut at the same intervals as before. The number of points at which bubbles of gas emerged at the cut surface were observed. The results obtained by this method were essentially the same as those with mercury when duplicated on the same shoots. By varying the pressure from 0.4 atmosphere to 1.5 atmosphere of either gas or mercury, no change in the results was obtained. Fig. 2 shows the device used in the second method.

Due to the heterogeneity of the material, which was also realized by Farmer (11), a modification of his statistical method was used in presenting the results of the work.

B. The area of the cross-section of tracheae and wood.

From the above observations, apple (Delicious variety) and prune (French variety) shoots were considered good material for study of the distribution, size, and the relative area of the vessels. Four shoots, 75 cm. in length, were cut from each of the apple and prune trees used previously. These were a few of the shoots which had been kept under observation from the appearance of the leaves until the time of cutting, in order that any leaf which might fall could be observed. Immediately after cutting, leaves were stripped off and blue prints made in such a manner as to show the distribution of leaves for every 15 cm. By this means the number and area of leaves in every 15 cm. could be separately determined. Afterwards, from these blue prints, the area was calculated by means of a planimeter. Cross-sections of the shoots were cut at every 15 cm. up to 75 cm., stained and mounted according to the directions of Chamberlain (1) and Langdon (23).

Before making the sections, the total water conductivity of each of the 15 cm. long pieces was measured under standard conditions as used by Farmer (11, 12) and Holmes (17, 18) in their studies of water conductivity. Distilled water was used for this purpose. It is true that cutting may bring air into the tracheae as shown by Hartig (15). Furthermore, during transpiration there must be continuous tracts of tracheae which are free from air for a considerable distance in cross-section as stressed by Dixon (6). In order to secure the typical stream generally assumed to be present in plants in nature, water was allowed to run for five minutes before any record was taken. About three sections from each level of each shoot were examined under a microscope. Lumina of the tracheae, from four to six selected areas from each year's wood, out of each of the three sections from every cut, were outlined with a calibrated microscopic equipment on millimetre-squared paper. The locations on the slides were selected at random. The areas occupied by the outlines of the lumina of tracheae, as well as the selected areas of the wood, were measured by a planimeter and checked by the millimetre squares of the paper. All areas were then reduced to actual areas, including that of the cross-section, and were calculated in square mm. Close agreement between the areas as determined by the squared paper, and as measured by the planimeter, was assumed to give accuracy enough for this study.

C. Determination of the age of the wood concerned in conduction.

A part of the data obtained from B above with reference to the area of tracheae in each year's growth suggested that the current year's wood might be sufficient for conduction. Conclusions drawn from Rumbold's (29) studies on chestnut and Elliot's (9) observations on apple and willow

agreed more or less with this theoretical possibility. However, Rumbold and Elliot used the boring method which allows air to get into the tracheae, as shown by Dixon (6), who says 'when a branch is cut even under water, it is possible that bubbles are formed in the tracheae by the act of cutting'.

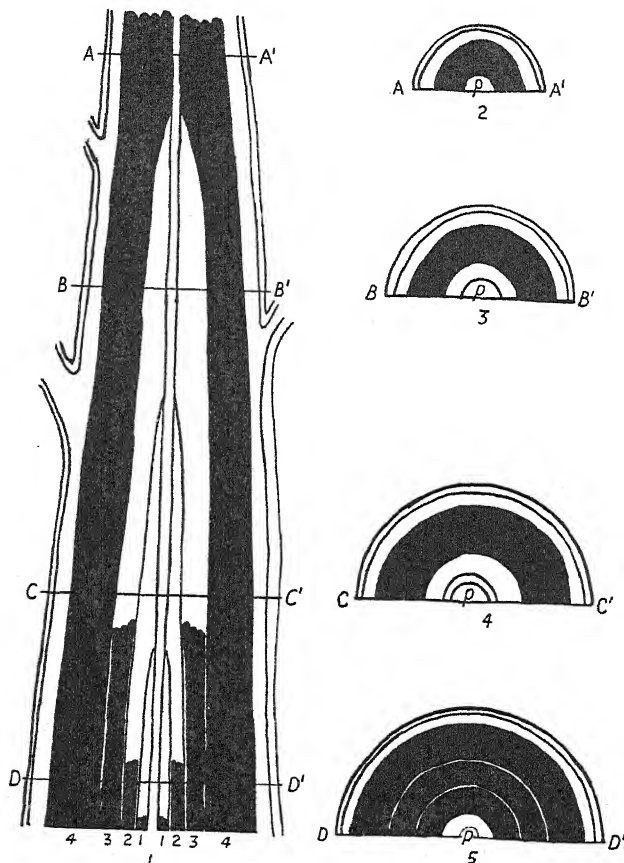


FIG. 3. (1) Longitudinal section of four-year-old willow tree. Figures 1, 2, 3, and 4 at the bottom indicate wood formed in the 1st, 2nd, 3rd, and 4th year. Solid black shows the route of dye as far as it was conducted. Note the stoppage of dye below C-C' in all but the 4th year's wood. Only the 4th year's wood transported dye from the base to the apex. Also note the width of each year's wood. Pith is shown in the centre. 2, 3, 4, and 5 are cross-sections at various levels of the tree, as indicated. Note that in all sections but the last (D-D') dye appeared only in the last-formed wood. 'P' indicates pith in all the sections.

He also pointed out that the amount of water transmitted in the stream would be affected by the number of tracheae, which contained bubbles, and which would be put out of action for the transmission of water. Such a complication, due to air and other factors, produced by the boring, is not typical in the natural process of the ascent of sap. In these experiments to determine whether all the wood or a particular year's wood conducts water in higher plants two four-year-old willow trees were selected near

Camp D, Montezuma Ranch No. 179, belonging to the California Packing Corporation, Rio Vista, California. To avoid the entrance of air into the tracheae, the willow trees were dug with as little injury to the roots as possible, washed with water under pressure, and the entire tree put into large cans full of approximately 0.5 per cent. congo red solution and allowed to stand for 90 hours. The whole tree was divided longitudinally. From one half, cross-sections at four different points from near the apex to the base (above the roots) were prepared (Fig. 3). The other half was first cut into pieces one foot in length, each of which was cut longitudinally in as thin but continuous strips as was possible with a razor.

These sections were then run through 20, 30, 40, 50, 70, 80, 95, and 100 per cent. alcohol followed by a xylol series, and finally dipped in cedar oil, following Dr. Kraus' method as given by Chamberlain (1) and Chandler (2). These sections were so transparent that the path of the conduction of the dye used (congo red) could be accurately followed without the use of a microscope. The information gained by this study will be presented in an appropriate place.

DISCUSSION AND PRESENTATION OF THE DATA.

A. *Maximum length and number of tracheae.*

The data presented in Table I, column 3, shows that the maximum length of the tracheae in different shoots of the same species lies within a narrow range, which is quite constant for the species. The different kinds of shoots studied, however, vary among themselves as to maximum length of tracheae. For instance, the maximum length of tracheae in the Delicious apple is about 32 ± 1 cm. (with the exception of shoots numbers 6, 7), in Bartlett pear it is about 16 ± 2 cm. (except shoots numbers 2, 3, 4), in quince it is about 20 ± 3 cm., in French prune about 36 ± 2 cm., in apricot about 45 ± 5 cm., in almond about 32 ± 4 cm., in *Vitis vinifera* 51 ± 1 cm. (except cane 1), in *V. Labrusca* about 14 ± 1 cm. (except 1, 6, 8), in peach 39 ± 3 cm., in plum 42 ± 4 cm., in cherry 20 ± 3 cm., and in walnut 18 ± 3 cm.

The tracheae in the shoots of drupe-bearing trees studied (except cherry) are longer than tracheae in the shoots of apple, pear, and quince. The average length of the former is 40 cm., while in the latter it is 23 cm. There is a great variation in the length of the tracheae of the two varieties of grapes studied. Whereas *Vitis vinifera* cane has the longest tracheae (51 ± 1 cm.), *V. Labrusca* (14 ± 1 cm.) has the shortest of all the shoots studied (Fig. 4).

TABLE I.

Data showing Maximum Length of Tracheae and Number of Tracheae at the Base of the Fruit Shoots, as seen by Mercury Points.

1. Kind of shoot.	2. Shoot No.	3. Max. length of tube in cm.	4. Diameter in cm. tip base.	5. No. of tracheae at the base.	6. Total length of shoots in cm.
Apple (Delicious) .	1.	33.5	0.78 1.0	57	99.0
	2.	31.0	0.79 0.95	56	91.0
	3.	34.0	0.76 1.0	53	100.0
	4.	34.0	0.76 1.0	52	103.0
	5.	31.5	0.78 0.99	51	96.0
	6.	17.0	0.69 0.88	42	78.0
	7.	15.0	0.84 1.0	44	72.0
	8.	34.1	0.76 0.99	53	107.0
	9.	33.6	0.77 1.1	58	91.0
Pear (Bartlett) .	1.	15.0	0.76 0.8	28	60.0
	2.	27.0	0.70 0.93	49	80.0
	3.	5.0	0.84 0.86	11	51.0
	4.	30.0	0.78 0.88	5	74.0
	5.	13.0	0.83 0.9	8	68.0
	6.	18.0	0.56 1.1	35	79.0
	7.	14.0	0.91 0.99	36	74.0
	8.	16.0	0.81 1.0	35	72.0
	9.	16.5	0.80 1.1	39	76.0
	10.	15.0	0.90 0.99	36	62.0
	11.	17.2	0.88 0.98	37	73.5
Quince . . .	1.	23.0	0.82 0.93	44	86.0
	2.	20.0	0.82 0.9	27	83.0
	3.	8.5	0.72 0.8	15	45.0
	4.	21.0	0.82 0.92	40	82.0
	5.	21.0	0.83 0.91	29	73.0
	6.	22.0	0.79 0.90	32	75.0
	7.	24.0	0.81 0.93	35	79.0
Prune (French) .	1.	38.5	0.78 1.2	113	97.0
	2.	38.0	0.8 1.0	111	86.0
	3.	27.0	0.69 1.3	186	110.0
	4.	16.0	0.74 0.99	110	74.0
	5.	31.0	0.89 1.0	109	73.0
	6.	35.0	0.78 1.0	106	60.0
	7.	36.5	0.9 1.0	110	60.0
	8.	36.0	0.78 1.0	105	56.0
	9.	37.0	0.76 1.0	109	62.0
	10.	37.5	0.77 1.2	113	78.0
	11.	37.2	0.76 1.1	118	60.0
Apricot . . .	1.	45.0	0.56 0.89	35	61.0
	2.	50.0	0.59 0.82	44	88.0
	3.	44.9	0.58 1.0	42	66.0
	4.	38.0	0.73 1.0	42	73.0
	5.	49.0	0.60 1.0	44	82.0
	6.	47.0	0.62 1.1	47	80.0
Plum . . .	1.	36.0	0.73 0.94	48	45.0
	2.	46.0	0.75 1.3	57	75.0
	3.	47.0	0.7 0.97	58	96.0
	4.	42.0	0.75 1.2	51	96.0
	5.	45.0	0.76 1.1	53	90.0
	6.	45.0	0.75 1.1	52	95.0

1. Kind of shoot	2. Shoot No.	3. Max. length of tube in cm.	4. Diameter in cm. tip base.		5. No. of tracheae at the base.	6. Total length of shoots in cm.
Peach	1.	42.5	0.71	0.95	82	75.0
	2.	36.0	0.69	1.1	64	58.0
	3.	46.5	0.64	0.93	62	82.0
	4.	42.0	0.58	1.05	53	77.0
	5.	32.0	0.7	1.1	52	72.0
	6.	28.0	0.78	0.97	14	77.0
	7.	51.2	0.6	1.05	55	79.0
Cherry	1.	13.0	0.76	0.92	7	60.0
	2.	22.5	0.8	0.9	14	76.0
	3.	25.0	0.8	1.1	31	76.8
	4.	21.0	0.75	1.0	23	68.0
	5.	21.0	0.78	1.0	22	64.0
	6.	21.5	0.76	1.1	23	65.0
Walnut	1.	6.0	0.87	0.92	11	70.0
	2.	18.5	0.90	1.0	20	58.0
	3.	20.0	0.87	1.1	31	91.0
	4.	21.0	0.98	1.1	35	98.0
	5.	15.5	0.8	0.85	7	71.0
	6.	17.3	0.82	0.87	13	75.0
Grape. (<i>V. vinifera</i>)	1.	84.0	0.97	1.45	78	100.0
	2.	51.0	0.75	1.2	55	84.0
	3.	51.0	0.81	1.1	61	84.2
	4.	51.0	0.80	1.2	67	84.0
	5.	52.0	0.82	1.1	63	84.0
	6.	51.0	0.81	1.1	66	72.0
Grape. (<i>V. Labrusca</i>)	1.	38.0	0.8	1.1	24	120.0
	2.	14.0	0.77	0.92	6	100.0
	3.	13.0	0.79	1.0	11	80.0
	4.	14.0	0.78	1.1	9	100.0
	5.	15.0	0.8	1.1	12	100.0
	6.	17.0	0.82	1.2	11	101.0
	7.	6.0	0.85	0.9	12	73.0
	8.	7.0	0.87	0.92	11	78.0
Lemon (Eureka)	1.	24.0	0.84	0.9	9	72.0
	2.	27.0	0.86	0.92	11	74.0
Orange (sour)	1.	42.0	0.76	0.93	20	75.5
	2.	31.0	0.55	0.85	22	63.0
	3.	35.0	0.67	0.89	23	67.0

All shoots in this table are of the same age. The tip diameter as here given is the diameter at that distance behind the tip where the presence of tracheae was first shown by the presence of mercury drops.

The maximum length of the tracheae in Citrus shoots is about that of the stone fruits, although not identical. Since very few Citrus shoots were used in this study, and since the trees from which these shoots were obtained were not vigorous, this data is merely indicative.

A study of columns 3 and 6 seems to indicate, in general, that there is a decided relationship between the maximum length of the tracheae and the total length of the shoots. For any species studied, among twigs of equal

age, the longer shoots have tracheae of greater length. Whether longer tracheae depends upon the greater elongation of the shoot, or the longer shoots can arise because of longer tracheae, we are not yet in a position to determine.

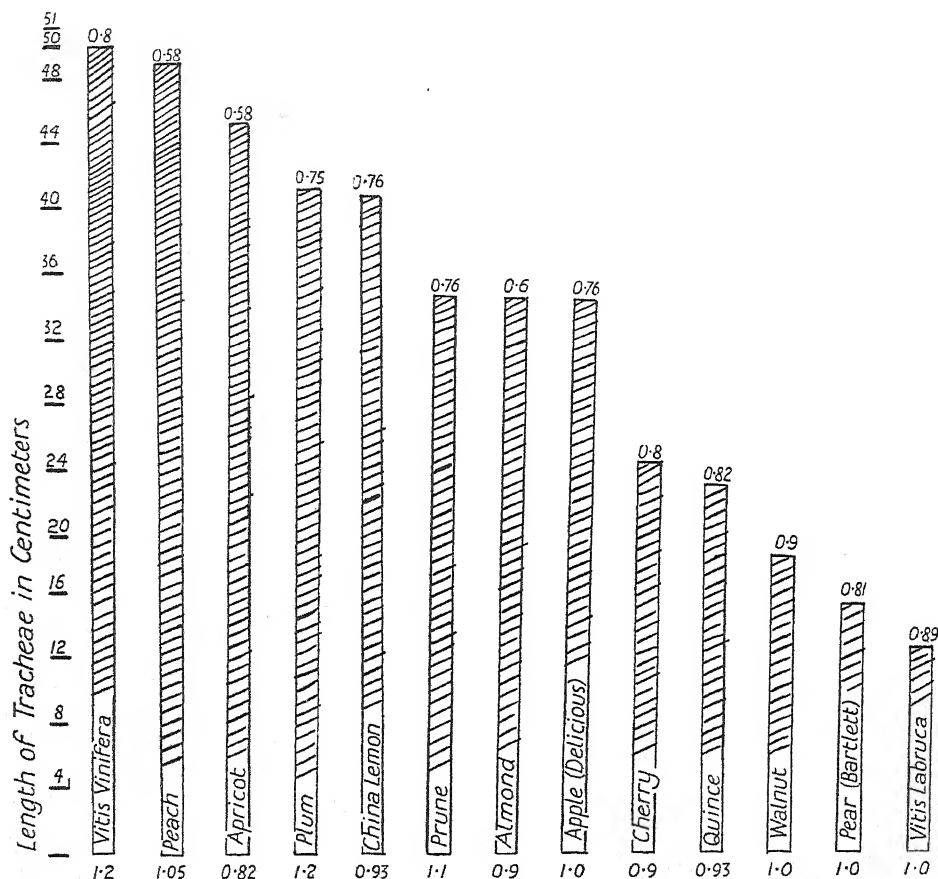


FIG. 4. Chart showing comparative maximum lengths of tracheae in various shoots. Figures at the top indicate diameter of apex when tracheae first appeared. Figures at the base indicate diameter of base of shoot.

A study of columns 3 and 4 reveals an absence of correlation between the diameter (either at the top or the base of the shoot) and the maximum length of the tracheae or the length of the shoot. However, there is a marked correlation between the number of tracheae and the diameter of the shoot. Columns 4 and 5 show this relationship between the diameter of a twig at the base and the number of tracheae at the same point. In general, it seems that as the diameter of a shoot increases the number of the tracheae increases also. Further evidence of this kind is brought

out by Fig. 5. The table shows no direct relationship between the total length of the shoot and the number of tracheae, nor does the number

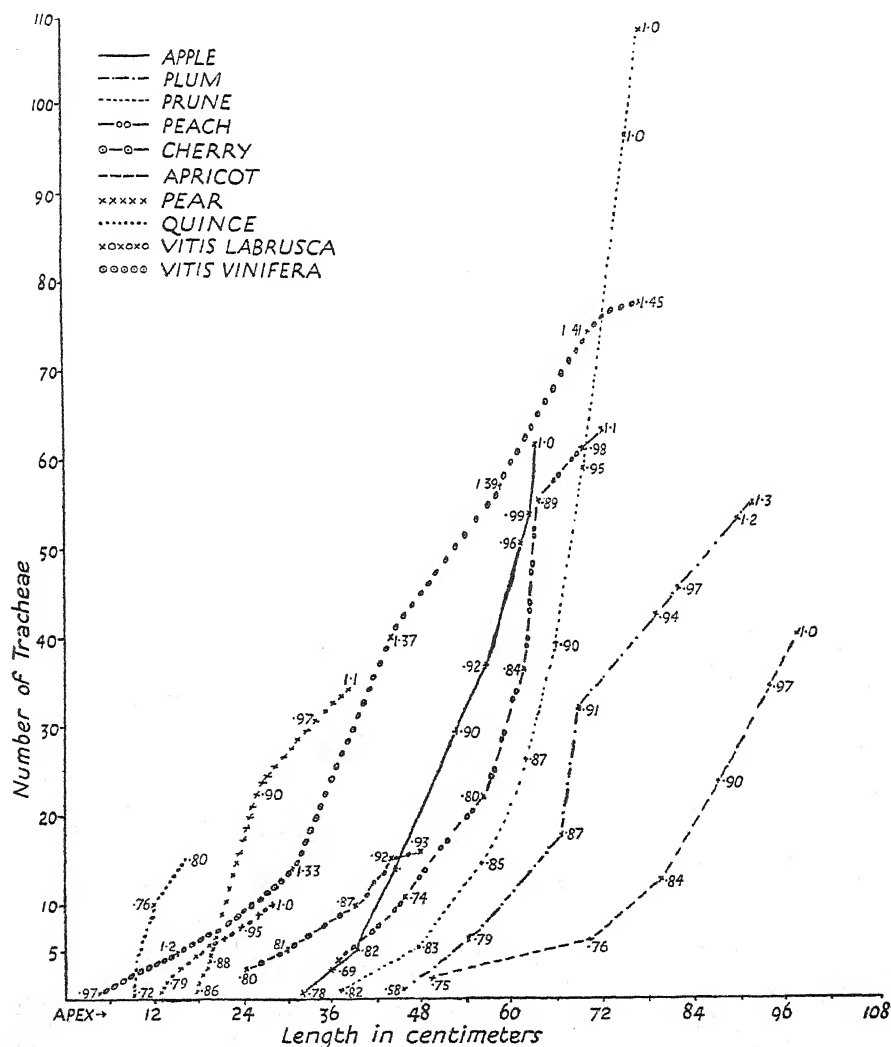


FIG. 5. The distribution of tracheae at different levels as seen in cross-sections of various shoots. Figures on the curves indicate the diameter of the shoot at the levels where the tracheae were counted.

of tracheae present seem to be as constant for the species as is the maximum length of the tracheae. Fig. 5 shows the number of tracheae as seen in cross-sections at different levels of the shoots studied. It brings out clearly that with increasing diameter of an individual shoot the number of tracheae increases.

TABLE II.

The Absolute and Percentage Cross-section Area of the Wood and the Lumina of Tracheae at Various Levels of Apple and Prune Shoots.

All Areas are expressed in square millimetres

Kind and No. Shoot.	Age in Years.	Cm. below the Apex.	Absolute Area of the						Absolute Area of Tracheae in				Calculated Area of Tracheae per 100 sq. mm. of Wood in —			
			Pith.	Third Year's Wood.	Second Year's Wood.	First Year's Wood.	Total Wood.		Third Year's Wood.	Second Year's Wood.	First Year's Wood.	Total Wood.	Third Year's Wood.	Second Year's Wood.	First Year's Wood.	Total Wood.
1. Apple (Delicious).	2.	3.	4.	5.	6.	7.	8.		9.	10.	11.	12.	13.	14.	15.	16.
	1	1	1.01	0.437	—	—	0.437		0.065	—	—	0.065	14.9	—	—	14.9
	1	15	1.45	3.0	—	—	3.0		0.548	—	—	0.548	20.1	—	—	20.1
	2	30	2.90	6.3	1.12	—	7.42		1.45	0.167	—	1.617	23.0	14.0	—	21.8
	2	45	1.22	8.12	1.7	—	9.82		3.8	0.49	—	4.29	46.7	13.5	—	43.7
	3	60	1.22	10.2	4.15	—	14.35		3.8	0.74	—	4.54	37.3	17.8	—	31.8
	3	75	6.8	15.12	3.5	10.13	28.25		4.97	0.83	2.33	8.13	33.0	23.7	23.0	34.3
	1	1	1.01	0.52	—	—	0.52		0.074	—	—	0.074	14.2	—	—	14.2
	1	15	1.45	3.88	—	—	3.88		0.698	—	—	0.698	18.0	—	—	18.0
	2	30	1.45	3.84	0.965	—	4.805		1.08	0.155	—	1.235	28.1	16.1	—	25.6
	2	45	0.82	11.35	0.87	—	12.22		3.3	0.194	—	3.494	29.1	22.1	—	28.5
	3	60	1.45	14.0	2.18	3.88	17.06		3.77	0.381	0.572	4.023	27.0	17.5	14.5	23.8
	3	75	4.88	20.4	3.39	11.5	35.29		7.17	0.885	3.47	11.52	52.5	26.1	33.6	32.4

Apple (Delicious).										Plum (French Prune).														
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18.1	—	—	—	18.1
1	1	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	19.1	—	—	—	19.1
2	2	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	24.6	—	—	—	24.6
2	2	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	18.3	—	—	—	18.3
3	3	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	20.0	20.0	26.1	32.9	29.9
3	3	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	32.4	30.4	38.4	48.8	32.9
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15.8	—	—	—	15.8
1	1	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	19.1	—	—	—	19.1
2	2	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	23.1	—	—	—	23.1
2	2	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	29.2	—	—	—	29.2
3	3	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	20.0	20.0	26.1	35.3	48.8
3	3	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	26.1	26.1	30.4	35.3	35.3
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15.0	—	—	—	15.0
1	1	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	24.0	—	—	—	24.0
2	2	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	29.2	19.2	27.8	35.3	28.4
2	2	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	33.1	17.8	29.1	35.3	29.4
3	3	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	35.3	20.7	24.3	30.4	27.0
3	3	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	35.4	22.6	27.2	45.5	45.5
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17.3	—	—	—	17.3
2	2	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	34.2	24.1	32.3	45.5	33.0
3	3	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	37.2	26.0	29.9	35.3	32.8
3	3	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	51.6	5.6	39.9	48.8	30.9
3	3	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	56.4	14.4	52.2	60.8	50.8
3	3	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	62.2	14.4	52.2	70.8	60.8
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16.2	—	—	—	16.2
2	2	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	29.0	—	—	—	27.9
3	3	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	33.0	21.0	25.0	32.9	36.4
3	3	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	42.0	24.0	34.0	42.0	38.0
3	3	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	44.0	13.1	32.2	40.8	35.0
3	3	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	45.9	19.0	39.1	48.8	39.0

Figs. 4 and 5 combined seem to show that there may or may not be a correlation between length and diameter of a shoot.

A. Area occupied by the tracheae and the wood.

The data obtained from the cross-sections of the apple and prune shoots are presented in Table II. Column 8 shows that the total absolute area of wood increases from the apex toward the base. Third year's wood (column 5) shows the same general trend as the total wood, but the second year's (column 6) does not make any great increase, although the general trend is like the total wood. Comparing columns 5, 6, and 7, it seems that the third year's wood makes the largest increase throughout the shoot, since, both in apple and prune shoots, the major wood area lies in the third year's growth. This is true of all shoots.

The total area occupied by the lumina of tracheae (columns 9, 10, 11, and 12) increases from the top to the base in much the same way as the area of wood, already discussed. The absolute area of tracheae in the third year's wood increases (column 9) from apex to base of the twig, while tracheae in the second and the first year's wood (columns 10 and 11) may or may not increase. When apple and prune shoots are compared, it will be found that there is more total wood in the apple shoots than in the prune at a given level below 15 cm. from the apex. As shown by the data, the same holds true for the absolute area occupied by the lumina, those of the apple tracheae at any level of the shoot below 15 cm. being greater than those of the prune shoot at the same level. However, the percentage of the total area occupied by the lumina of the tracheae of prune wood is much greater than the percentage area occupied by the tracheae of apple wood below 15 cm., as indicated by columns 12-16 of Table II. In other words, there is a greater area of the lumina of tracheae per 100 sq. mm. of wood in the prune shoot than in the apple shoot at the same distance below 15 cm. However, at any individual point between 1 and 15 cm. below the apex, the percentage area occupied by the lumina of the tracheae in both kinds of shoots is about the same. At the base the difference is very significant, as shown by the data.

When the development of the tracheae is followed qualitatively from the top to the base in both the apple and the prune wood, it is found, as shown in Fig. 6, that at 1 cm. below the tip prune wood has tracheae of smaller size than the apple wood. Thus, area of tracheae per unit area of apple wood at this point, is greater than the area of tracheae per unit area of prune wood at the same level.

At 30 cm. below the apex this relationship is reversed, i. e. the area occupied by the lumina of the tracheae of prune is greater than that of the apple per unit cross-section of wood. At 60 cm. below the apex the area

of tracheae per unit area of the prune wood is distinctly greater than that of the apple (Fig. 6, C, F). It seems to agree with the data obtained by the measurements as presented in column 16 of Table II, as well as in Table III. The latter table also seems to indicate that, at a

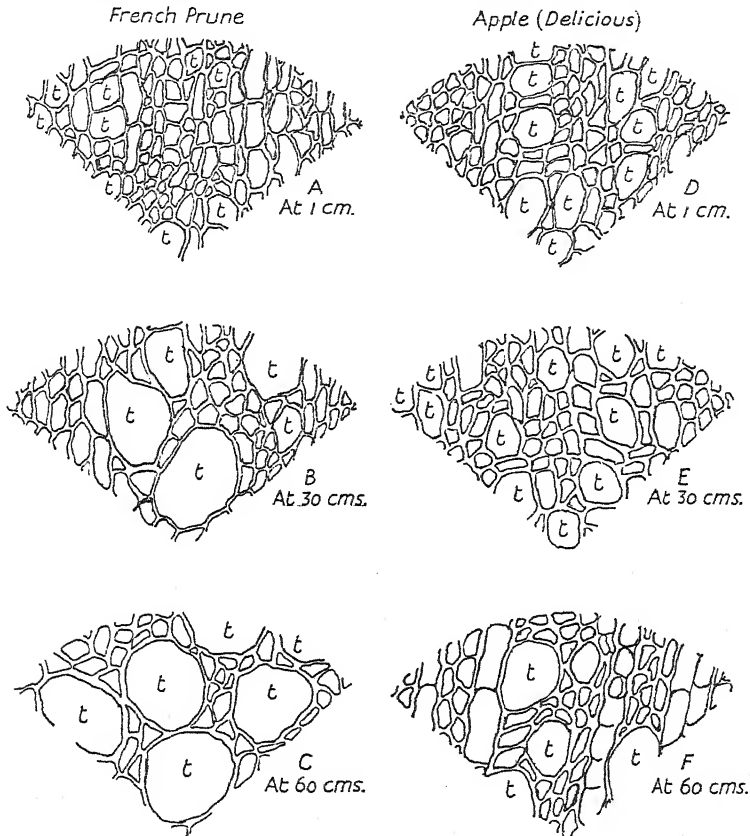


FIG. 6. Cross-sections of the last year's apple and prune wood. The sectors represent a unit surface of the sections, drawn at the same magnification ($\times 440$) but at different levels of the same shoots. A, B, and C show sectors of the apple wood at 1, 30, and 60 cm. below the apex respectively, while D, E, and F illustrate the same view in prune. Tracheae are indicated by 't'.

definite point throughout the length of a shoot, the absolute and the percentage area of the cross-section occupied by the lumina of tracheae in the third year's wood are greater than those of the second year, and those of the second year greater than those of the first year. This holds true of both the apple and the prune shoots. Furthermore, the total percentage area of the lumina of the tracheae in the apple and prune shoots increases about twice and three times, respectively, from the top to the base. Column 4 of Table II indicates that the cross-section area of the pith varies at different levels of the shoot. There is no progressive increment of the pith

area from the apex to the base. However, in both the apple and prune shoots, there is a maximum area of the pith, for the age of twigs studied, at 75 cm. below the apex. It may be noted that, during the preparation of sections for this study, the tissues had to be dehydrated with alcohol. Under such

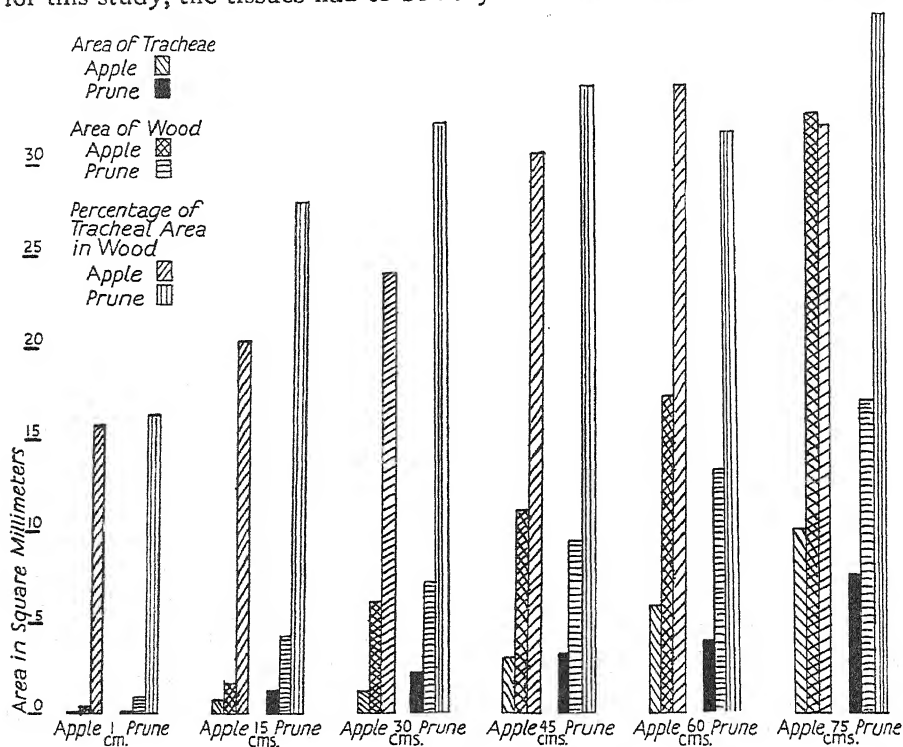


FIG. 7. Chart showing the relationship between absolute and percentage area of tracheae and wood in French prune and apple (Delicious) shoots.

a treatment shrinkage from 10 to 13 per cent. was noted when measured under the microscope and compared with similar sections not treated with alcohol. However, the shrinkage seems to be more or less uniform in all the sections, and throughout the surface of the same section; hence it is assumed that this factor would not cause an error in the relative values of the data presented in Tables II and III. The relationship between the absolute and the percentage area of the tracheae and the wood, as well as the importance of tracheae in the first, second, and third year's wood, has been illustrated by Figs. 7 and 8 respectively. It seems to me that these charts are self-explanatory. They seem to indicate the importance of the last-formed wood and of the area of lumina of tracheae therein, while Table III shows that, in general, there is a higher percentage of tracheae per unit of wood in prune than in apple. The points mentioned above have some correlation with their respective water conductivities, as is dis-

cussed in the following paragraphs. When the data from all the sections made during the growing season were summarised, it was found that the area of the lumina of tracheae in prune and apple was 50.2 and 28.7 per cent. of their respective woods. The summary is presented in Table III A.

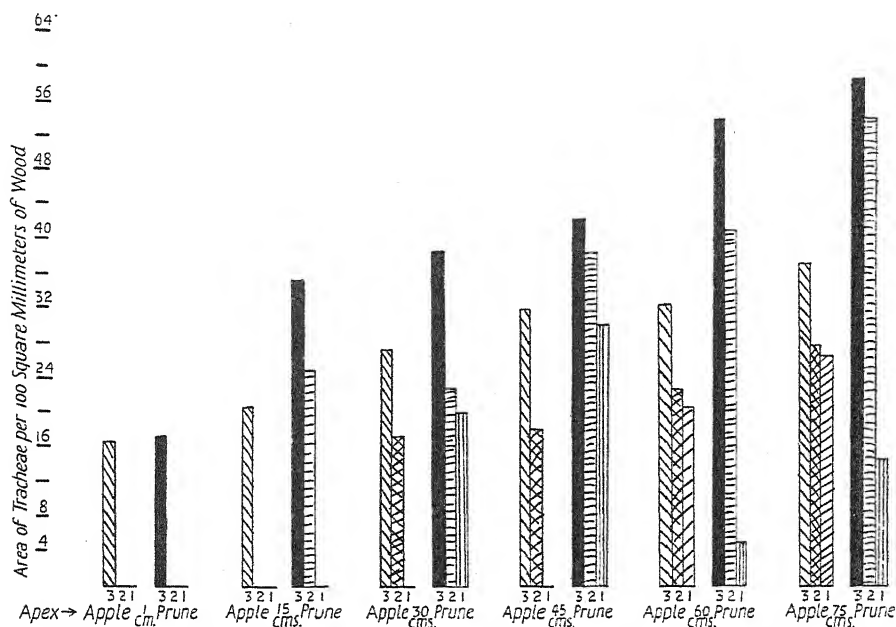


FIG. 8. Chart showing area of tracheae per square millimetre of one, two, and three years' wood at various levels of apple (Delicious) and French prune shoots. 1 = first year's tracheae, 2 = second year's tracheae, 3 = third year's tracheae.

C. The relationship between tracheae and leaves and their significance in water conductivity.

Data concerning the area of tracheae with reference to its conductivity per leaf has been presented in Table IV. Since in this table only number of leaves are recorded, it may be pointed out that in both apple and prune leaves a definite correlation has been found between the number and the area of the leaves, as measured with a planimeter from the blue prints. Furthermore, the average area of an apple leaf is found to be 2.51 times as great as the average prune leaf. This holds true for the leaves throughout the shoot, as well as within every 15 cm. of the length of the shoot.

Assuming that all wood conducts water, columns 4 and 6 of Table IV show that the tracheae in the last-formed wood (third year) alone have a capacity to carry 0.7 to 0.9 of the water required by the leaves for transpiration. In other words, the area of the lumina of tracheae in the third year's wood averages 70-90 per cent. of the tracheae of all the wood. The area

TABLE III.

Summary showing the Area occupied by Tracheae in 100 square mm. of Wood.

Kind of shoot.	Distance from the apex.	Age of the shoot at that distance.	Percentage of area occupied by tracheae in the			
			Third year's wood.	Second year's wood.	First year's wood.	Total wood.
1.	2.	3.	4.	5.	6.	7.
Apple (Delicious)	1	1 year	15.73	—	—	15.8
	15	1 "	19.04	—	—	19.1
	30	2 "	25.37	16.78	—	23.8
	45	2 "	30.67	17.33	—	29.9
	60	3 "	31.44	22.57	19.85	33.6
	75	3 "	35.5	26.73	27.1	31.3
French Prune	1	1 "	16.15	—	—	16.2
	15	2 "	29.1	19.85	—	27.7
	30	3 "	33.2	25.05	28.6	31.6
	45	3 "	42.4	33.15	23.85	33.4
	60	3 "	43.45	32.1	13.15	31.0
	75	3 "	45.9	38.7	18.5	45.1

Note.—Figures recorded in this table have been derived from the study of 432 different parts of 144 slides.

TABLE III A.

Percentage Area of Apple and Prune Woods occupied by Tracheae, from May to August, 1927.

Series number.	Month.	Kind of shoot.	% Area tracheae.
1.	May	Apple	26.4
"	"	Prune	47.9
2.	June	Apple	27.0
"	"	Prune	49.0
3.	July	Apple	28.6
"	"	Prune	50.0
4.	August	Apple	38.8
"	"	Prune	53.9
	Average	Apple	28.7
	"	Prune	50.2

Note.—These figures were derived from the study of 15 cross-sections (15 × 3 places) each month. The diameter of the shoots was about the same.

TABLE IV.

Data showing Area of Lumina of Tracheae and their Capacity to supply Water per leaf. (All areas are in sq. mm.)

Kind and no. of shoot.	Length of the shoot from apex to base.	No. of leaves above height in col. 2.	Area of lumina of tracheae in third year's wood.	Area of third year's tracheae per leaf.	Area of tracheae in the total wood.	Area of tracheae of the total wood per leaf.
I.	2.	3.	4.	5.	6.	7.
Apple I.	1 cm.	4	0.065	0.016	0.065	0.016
	15 "	10	0.548	0.055	0.548	0.055
	30 "	15	1.45	0.09	1.617	0.108
	45 "	20	3.8	0.19	4.29	0.214
	60 "	25	3.8	0.141	4.54	0.182
	75 "	29	4.97	0.151	8.13	0.28
II.	1 "	4	0.074	0.018	0.074	0.018
	15 "	11	0.698	0.063	0.698	0.063
	30 "	17	1.08	0.064	1.235	0.0726
	45 "	22	3.3	0.15	3.494	0.16
	60 "	27	3.77	0.14	4.023	0.149
	75 "	32	7.17	0.22	11.525	0.365
III.	1 "	4	0.235	0.058	0.235	0.058
	15 "	11	0.653	0.059	0.653	0.059
	30 "	16	1.41	0.088	1.568	0.098
	45 "	22	1.63	0.074	1.829	0.083
	60 "	30	3.5	0.116	4.382	0.161
	75 "	38	7.05	0.185	10.91	0.28
IV.	1 "	3	0.093	0.031	0.093	0.031
	15 "	9	0.70	0.078	0.70	0.078
	30 "	17	0.99	0.058	1.158	0.068
	45 "	22	3.18	0.145	3.37	0.153
	60 "	27	4.82	0.178	10.372	0.38
	75 "	32	6.76	0.211	11.20	0.35
Prune I.	1 "	8	0.133	0.0167	0.133	0.017
	15 "	27	0.463	0.0173	0.536	0.020
	30 "	41	1.23	0.03	1.855	0.045
	45 "	56	1.83	0.0327	3.186	0.055
	60 "	68	3.53	0.052	3.698	0.054
	75 "	78	3.67	0.047	8.08	0.104
II.	1 "	6	0.118	0.02	0.118	0.02
	15 "	22	1.04	0.047	1.163	0.053
	30 "	37	1.38	0.037	2.091	0.056
	45 "	51	2.42	0.047	2.772	0.054
	60 "	63	3.1	0.049	3.948	0.063
	75 "	75	4.92	0.065	8.282	0.111
III.	1 "	6	0.12	0.02	0.12	0.020
	15 "	26	0.868	0.033	0.970	0.037
	30 "	43	1.33	0.030	2.682	0.062
	45 "	61	1.9	0.031	3.215	0.053
	60 "	68	2.64	0.039	4.271	0.063
	75 "	82	3.91	0.047	6.05	0.073

of lumina of all the remaining wood is only 10–30 per cent. Columns 5 and 7 show that, whether the last-formed wood or the total wood is concerned in water conductivity, the area of the tracheae per leaf is greater in apple than in prune. If these figures be divided by 2.51 in case of apple, or multiplied by 2.51 in case of prune, in order to get equivalent units of both kinds, a unit area of apple leaf requires a larger area of the lumina of tracheae than a similar unit of prune leaf. In this an assumption is made that both apple and prune leaves have equal transpiring power per unit surface in unit time and under the same environmental conditions. Such a unit has been worked out by measuring the area of the tracheae in cross-section, the area of the leaves above the cut, and the conductivity of these tracheae in twenty apple and prune shoots of various sizes. From these data mean values have been calculated. In this connexion further data will be presented in the third part of this series. It seems surprising from the figures, that in spite of the heterogeneity of the shoots, the conductivity values per unit area are very constant. It was found that, under the above-mentioned standard conditions, it is theoretically possible for 0.738 sq. mm. tracheae of the prune wood to conduct the same amount of liquid as 1 sq. mm. tracheae of apple wood. This calculation is based on the assumption that the liquid conducted in both cases has the same viscosity. It has also been assumed that the transpiring power of apple and prune leaves, which is, of course, unlikely, see Curtis (5).

In order to gain some idea how much of the difference in the capacity of a unit area of the tracheae was caused by a variation in the transpiring power of the two types of leaves, their transpiration (per unit time under standard conditions) was measured by the cobalt chloride method, as originated by Stahl (33) and improved by Livingston and Shreve (24). An average of twenty readings of each kind of leaf was assumed to be satisfactory for this purpose. It was found that a unit surface of prune leaf transpired 10.1 per cent. faster per unit time than a unit surface of apple leaf under similar conditions. It has been shown previously that the amount of tracheal lumina required per unit area of leaf surface is, apple 1.0 and prune 0.738. As prune leaves transpire 110.1 units per unit of leaf area as compared with 100.0 units transpired by apple leaves per unit area, it is clear that the prune tracheae are still more efficient. The amount of lumina required for each unit of transpiration is, apple 1.0 and prune 0.670. The conducting capacities of the lumina of these tracheae is inverse of these numbers. In other words, a unit area of prune wood tracheae is 49 per cent. more efficient in its capacity to conduct water than a similar unit of apple wood, assuming that all water passing through the tracheae is transpired by the leaves, and assuming a constant ratio in the transpiration of the leaves of the two plants. If this is true, as indicated by the data so far presented, then we have to admit that area, length, and

number of the tracheae are not the only factors concerned in the conduction, but some other factor or factors, hitherto not studied, may offset the effects of the cross-section area of tracheae as a limiting factor. These results are in accordance with that of Inamdar and Shrivastava (20), who state: 'The diameter of the vessel is not the only contributing factor toward variations in the specific conductivity, as is illustrated by the fact that no quantitative proportionality is maintained between the diameter of the vessels on the one hand and the specific conductivity on the other.'

It may be pointed out that another variation, namely, length of the tracheae, has been eliminated in this case by measuring the area of tracheae between 1-15 cm. and also by measuring the conductivity of this range in both kinds of shoots. These measurements support the above statement, namely, that a unit area of tracheae in prune wood is 49 per cent. more efficient than in apple tracheae.

In the next paper (in its logical place) the factors concerned with this difference in favour of prune wood, will be discussed.

D. *Does all the wood take part in water conductivity?*

Fig. 3 illustrates diagrammatically the longitudinal and transverse sections of the willow trees, showing the areas concerned in the passage of congo red in wood above the roots. These sections seem to indicate that water and diluted substances (dyes) are transported in the last-formed wood, at least in willow. Some liquid undoubtedly passed in the first, second, and third year's wood upward, for some distance only, but the main continuous stream of the liquid (dyes) was transported in the fourth year's wood. Congo red passed from the base to the apex in the last-formed wood only. If the water is conducted in a manner similar to that demonstrated in this experiment, there seems little doubt that in woody plants the last-formed wood may be the route for the passage of water.

SUMMARY.

An attempt has been made to study the length, area, and number of tracheae in order to gain some idea of their role in the mechanism of water conductivity.

The following points seem to have been shown from the data:

1. The maximum length of the shoot and of the tracheae have some correlation, that is, the longer the shoot the longer the tracheae, but neither of them have any correlation with the diameter of the shoot.

2. The maximum length of tracheae varies considerably in the wood of different trees studied. Yet, in general, the length of the tracheae of various members of a group as drupes, pomes, and nuts, fall within a definite

range. In this respect an exception, as exhibited by canes of *Vitis vinifera* and *Vitis Labrusca*, has been pointed out.

3. There seems to be some correlation between the number of tracheae and the diameter of a shoot at a particular level. Since the diameter of a shoot gradually increases from the top to the base, the number of open tracheae also increase, as observed by the 'mercury points'.

4. Total wood and area of the tracheae increases from the top to the base of a shoot, as seen by cross-sections. Of this wood the last-formed wood increases by an appreciable amount.

5. Apple shoots have more total wood and tracheae area than prune below 15 cm. from the apex; but the percentage of the total wood area occupied by the lumina of tracheae at these points is greater in prune than in apple.

6. Pith area varies in cross-sections when followed from the top to the base of apple and prune shoots, although the maximum is reached at 75 cm., which is the basal point of the shoots studied.

7. The importance of the third year's wood in water conductivity is pointed out.

8. The area occupied by the lumina of tracheae of prune is 50.2 per cent. of the wood, while area of the lumina of apple is 28.7 per cent.

9. The importance of the area of the lumina of tracheae, rather than their number, is discussed, although at times the number of tracheae is of great significance in water conductivity.

10. Differences in the transpiring power of both apple and prune leaves have been determined by the cobalt-chloride method.

11. Theoretically, it is shown from the data that a unit area of the lumina of tracheae in prune wood is 49 per cent. more efficient in conducting water than a similar unit area of the tracheae in apple wood, assuming that the liquid to be carried in both kinds of wood has the same viscosity, and that other environmental conditions are identical.

12. From this study it is indicated, directly as well as indirectly, that only the last-formed wood is concerned in transporting water.

Sincere thanks are due to Dr. J. P. Bennett, Associate Professor of Pomology, University of California, for his help and encouragement during the course of this study.

The writer is also greatly indebted to Miss Katherine Washburn, of the North-Western University, Evanston, Ill., for her invaluable assistance in preparation of the drawings used in this paper.

Acknowledgement must be made for help rendered by Mr. Cheit Singh, then foreman of Camp D, Montezuma Ranch 179 of the California Packing Corporation, in connexion with the digging, packing, and hauling of the willow trees used in this study.

The writer is no less indebted to Dr. W. H. Chandler, Professor of Pomology, University of California, Berkeley, for the identification and use of his fruit trees in these experiments.

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The Sexuality of *Ascobolus stercorarius* and the Transportation of the *Oidia* by Mites and Flies.

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With Plate XIX and ten Figures in the Text.

I. INTRODUCTION.

ASCOBOLUS STERCORARIUS (Bull.) Schröt. (= *A. furfuraceus*, Pers.) is a coprophilous Discomycete of common occurrence on horse dung, cow dung, &c. It frequently appears on horse-dung cultures in the laboratory at Winnipeg and, on this account, it was chosen as material for an experimental study of sex in one of the Ascomycetes.

In 1920, Dodge (10), as a result of an experimental inquiry into the sexuality of *A. magnificus*, found that in this species the ascospores and the mycelia to which they give rise are unisexual and fall into two sexual groups, (+) and (-): when two (+) mycelia or two (-) mycelia are paired, they remain sterile; but, when a (+) mycelium is mated with a (-) mycelium, fruit-bodies are produced. In 1926, Betts (2) obtained similar results for *A. carbonarius*.

In 1930, after the work recorded in this paper had been completed, Ames (1) published a brief account of the results of his study of sex in ten species of *Chaetomium*, one species of *Fimetaria*, five species of *Pleuraea*, and one species of *Ascobolus*, *A. stercorarius*. He concluded that all these species are homothallic except the *Ascobolus*, which he regards as hetero-homothallic. He states that in *A. stercorarius*: 'Single spore cultures of this species gave rise to a few apothecia. By mating single spore cultures it was found that approximately fifty per cent. of the matings stimulated a great abundance of apothecia along the line where the mycelia of the two strains met.'

In what follows evidence will be adduced to show that *A. stercorarius* is not hetero-homothallic as Ames has supposed, but is heterothallic.

In the work about to be recorded an attempt has been made to determine not only (1) the sexual nature of the ascospores, but also (2) the sexual nature and function of the *oidia*.

In 1905, Claussen (6) observed that, in *A. stercorarius*, great numbers of oidia, in the form of chains, are produced on the mycelium derived from an ascospore. He germinated these oidia and obtained from them a mycelium which again produced oidia, and he succeeded in culturing the fungus in this way for one hundred successive generations; but in none of these generations did the mycelium produce any apothecia. The non-production of apothecia, as is indicated by experiments to be recorded in this paper, was doubtless due to the mycelia having been unisexual.

Professor A. H. Reginald Buller conceived the idea that the pycnidiospores of the Rust Fungi and the oidia of the Hymenomycetes, after transference from a haploid mycelium of one sex to a haploid mycelium of opposite sex, might germinate, and that the germ-tubes or mycelia so produced might unite with the haploid mycelium to which the pycnidiospores or oidia had been transferred and so bring about its diploidization. This suggestion has been verified experimentally: in 1927, by Craigie (7) for *Puccinia graminis* and *P. helianthi*; and, in 1931, by Brodie (4) for *Coprinus lagopus*. It has been shown by these observers that the pycnidiospores or oidia become effective diploidizing agents after transference either by hand or by insects.

Hitherto, no experimental work comparable with that of Craigie and of Brodie has been done with the oidia of the Ascomycetes; but, in what follows, it will be shown that in a typical Ascomycete, namely, *A. stercorarius*, the oidia function in the same way as those of the Hymenomycetes.

II. MATERIAL AND METHODS.

Fresh horse-dung balls were obtained from a stable and set in a large glass dish. After three or four weeks fruit-bodies of *A. stercorarius* appeared upon them. As soon as these fruit-bodies had attained maturity, a glass slide was suspended above them. In the course of a few minutes a considerable number of spores were discharged from the asci and many of them were shot on to the under side of the slide. Thus a spore-deposit was procured. By means of the dry-needle method (12) a number of the spores were removed one by one from the spore-deposit and were sown singly on the culture medium.

Trials were made with two kinds of culture media, horse-dung agar and fresh horse dung. The spores germinated equally well in both media, but the mycelium fruited better on the dung than on the dung agar. Dung agar was employed in the early stages of the work and dung exclusively in the later stages.

Dodge (8) found that many coprophilous species of the Ascobolaceae can be made to germinate by subjecting them to high temperatures, and he succeeded in germinating the spores of *A. stercorarius* in soil agar (to

which some sodium carbonate had been added) when the inoculated soil-agar plates had been heated in an oven for twenty minutes with the temperature rising to 65° C.

Spores of the Winnipeg material were placed in hanging drops of horse-dung agar at room temperature. About 25 per cent. of them germinated. Applying the method used by Dodge, an attempt was made to increase the number of spores which would germinate. The spores, in the form of a dry spore-deposit, were slowly heated under the electric-light bulb of a reading lamp until a thermometer placed beside them recorded the desired temperature, and then they were allowed to cool. The temperatures tried were 27.5°, 29°, 35°, 40°, 50°, 70°, and 80° C. It was found that heat above room temperature stimulated the spores to germinate and that the best result was obtained when the temperature was 35° C. Temperatures of 30°, 32.5°, 35°, 37.5°, and 40° C. were then tried, and the most satisfactory temperature for inducing germination was found to be 37.5° C. After the spores had been subjected to this temperature, 68 per cent. of them germinated. Similar results were obtained when the spores to be heated were first sown in hanging drops of dung agar. Throughout the course of the work the spores required for experiment were all heated to 37.5° C. either before being sown or just afterwards.

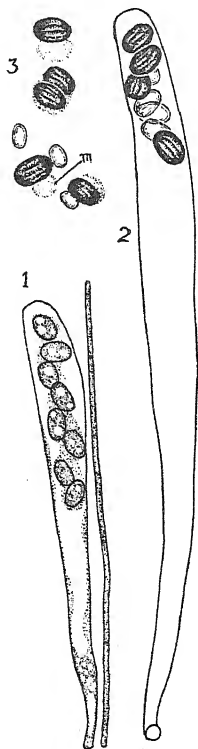
In the early part of the work, the spores were sown in hanging drops of dung agar and then each of the young mycelia was transferred to dung agar contained in a Petri dish. Later it was found better to sow the spores directly on the dung contained in small wide-mouthed bottles plugged with cotton wool.

The dung-agar plate method for cultivating the mycelia was abandoned as soon as it was observed that the plates had become invaded by mites which had wandered to them over the surface of the laboratory table from an old unsterilized horse-dung culture. No mites ever obtained access to the bottles plugged with cotton wool. As will be shown later, it is possible for mites to carry oidia from one dung-agar plate to another in a pile of plates under the same bell-jar and, therefore, for accurate investigations on sexual phenomena in species of fungi-bearing oidia it is of the utmost importance that mites should be excluded from the cultures.¹

The spores of *A. magnificus*, according to Dodge (9), are normally rose-purple or violet, but in certain of his cultures the apothecia produced and ejected colourless spores only. In *A. stercorarius*, as grown by the writer in a moderately lighted room, nearly all the ripe asci contain not

¹ Thom and Church (14), in their treatise on the Aspergilli, state that mites, which in size are commonly at the limit of visibility for the unaided eye, 'have passed unnoticed for considerable periods by persons who are otherwise good culture workers. . . . To reach an attractive food supply a mite will frequently go through a cotton plug as ordinarily made and occasionally seems to get through even a paraffined plug. As a factor in the mixing of strains in a laboratory collection, mites must not be ignored.'

only normal spores which are brown, furrowed, and relatively large, but also some abnormal spores which are colourless, smooth, and usually of smaller size (Fig. 2), and in spore-deposits (Fig. 3) the colourless spores



TEXT-FIGS. 1-3. Asci and ascospores of *Ascobolus stercorarius*. $\times 275$. 1. Young ascus and paraphysis, ascus containing immature spores. 2. Old ascus about to discharge its spores. It contains four larger brown spores and four smaller colourless spores. 3. A deposit of eight spores which have been shot out of a single ascus on to a glass slide. There are five brown spores and three small colourless spores. The normal coloured spores are attached to the glass by the mucilage, *m*, derived from the outer cell-wall.

make up about 37 per cent. of the whole number. Colourless spores were sown in dung agar, but none of them germinated.

Spores which have been sown on horse dung germinate within a few hours and, in the course of two days, the mycelium derived from the germ-tube becomes extensive and can be seen with the naked eye. At the end of two days after a spore has been sown, the mycelium begins to develop aerial hyphae which project from the substratum and become divided up into chains of oidia. These oidial hyphae appear macroscopically as a delicate white fluff.

The chains of oidia, when required for experiment, were removed from the mycelia of monosporidial origin with the help of a needle. This was drawn through the aerial mycelium, which was pulled away like cobweb.

III. THE SEXUALITY OF THE ASCOSPORES.

To determine the sexuality of the ascospores, two sets of experiments were made. In the first set the mycelia were paired on horse dung and in the second set on horse-dung agar.

	1	2	3	9	10	4	5	6	7	8
1	—	—	—	—	—	+	+	+	+	+
2	—	—	—	—	—	+	+	+	+	+
3	—	—	—	—	—	+	+	+	+	+
9	—	—	—	—	—	+	+	+	+	+
10	—	—	—	—	—	+	+	+	+	+
4	+	+	+	+	+	—	—	—	—	—
5	+	+	+	+	+	—	—	—	—	—
6	+	+	+	+	+	—	—	—	—	—
7	+	+	+	+	+	—	—	—	—	—
8	+	+	+	+	+	—	—	—	—	—

TABLE I. *Ascobolus stercorarius*. All possible pairings of ten mycelia each derived from a single ascospore. Pairings on horse dung.

(1) *Mycelia paired on horse dung*. Single ascospores were transferred to hanging drops of horse-dung agar and then heated to 37.5° C. The first ten spores that germinated were used to inoculate as many small wide-mouthed bottles of dung. The ten monosporous mycelia grew well and, in a few days, were ready for use in mating experiments. Pieces of each mycelium, together with scraps of the dung on which they grew, were removed from the ten bottles and were paired in all possible combinations on sterilized horse dung in a series of other bottles.

The criterion of sex employed has been the production or non-production of fruit-bodies. In a combination of two monosporous mycelia, if fruit-bodies are produced, then the mycelia are regarded as being of opposite sex, but if the combination remains sterile, then the mycelia are regarded as being of one and the same sex.

The results of the pairings of the ten monosporous mycelia are embodied in Table I. The ten mycelia were assigned the numbers 1 to 10. A (+) sign indicates that the combination concerned produced apothecia, and a (—) sign that it remained sterile. The original table of results has been re-arranged so as to bring like mycelia together.

A survey of Table I shows that the mycelia Nos. 1–10 fall into two

sexually opposite groups: Nos. 1, 2, 3, 9, and 10 are of one sex and Nos. 4, 5, 6, 7, and 8 are of the other and opposite sex.

In each one of the fertile combinations, the apothecia were large and

	11	13	14	17	19	20	12	15	16	18
11	—	—	—	—	—	—	+	+	+	+
13	—	—	—	—	—	—	+	+	+	+
14	—	—	—	—	—	—	+	+	+	+
17	—	—	—	—	—	—	+	+	+	+
19	—	—	—	—	—	—	+	+	+	+
20	—	—	—	—	—	—	+	+	+	+
12	+	+	+	+	+	+	—	—	—	—
15	+	+	+	+	+	+	—	—	—	—
16	+	+	+	+	+	+	—	—	—	—
18	+	+	+	+	+	+	—	—	—	—

TABLE II. *Ascobolus stercorarius*. All possible pairings of ten mycelia each derived from a single ascospore. Pairings on dung agar.

abundant and they appeared between the fifth and the eighth day after the combination had been made. On the other hand, each sterile combination showed no sign of fruiting even after it had been kept for six weeks.

Stock cultures of all the monosporous mycelia, Nos. 1–10, were kept moist in bottles for four months, yet in none of them did any apothecia appear. The sterility of all these control cultures is in accordance with the view that a monosporous mycelium is strictly unisexual and that the development of apothecia in a combination of two mycelia is due to a sexual reaction between mycelia of opposite sex.

(2) *Mycelia paired on dung agar*. Ten new monosporous mycelia, Nos. 11–20, were obtained by germinating as many spores, and then they were transferred to ten Petri dishes containing dung agar. As soon as the mycelia were large enough, pieces of each of them, together with the sub-jacent agar, were removed from the dishes and were paired in all possible combinations on sterilized dung agar in Petri dishes.

The results of the pairings of the ten monosporous mycelia are embodied in Table II. Again a (+) sign indicates that the combination of two mycelia produced apothecia, and a (–) sign that it remained sterile. As before, the original table of results was re-arranged so as to bring like mycelia together.

From an inspection of Table II, it will be seen that the mycelia Nos.

11-20 fall into two sexually opposite groups: Nos. 11, 13, 14, 17, 19, and 20 are of one sex and Nos. 12, 15, 16, and 18 are of the other and opposite sex.

In each of the fertile combinations, the apothecia, as compared with those obtained in the first series of experiments, were very small, very few in number, and much later in making their appearance. Whereas in the first series of experiments the apothecia developed from the fifth to the eighth day, in the second series the apothecia developed from the tenth to the seventeenth day. It is clear that horse dung is a far more suitable medium for experiments on sex with *A. stercorarius* than dung agar.

The two series of experiments recorded above clearly indicate that in *A. stercorarius* the ascospores and mycelia of monosporous origin are unisexual and that the production of fruit-bodies is dependent on the co-operation of two mycelia of opposite sex. In other words, *A. stercorarius* is heterothallic.

IV. THE SEXUALITY OF THE OIDIA.

It is known that oidia occur in several species of *Ascobolus*. Thus those of *A. denudatas* have been illustrated by Brefeld (3) and those of *A. lignatilis* by Falck (11).

In *A. stercorarius* oidia are constantly and abundantly produced by all mycelia of monosporous origin, and they continue to be produced by two mycelia of opposite sex which have been mated.

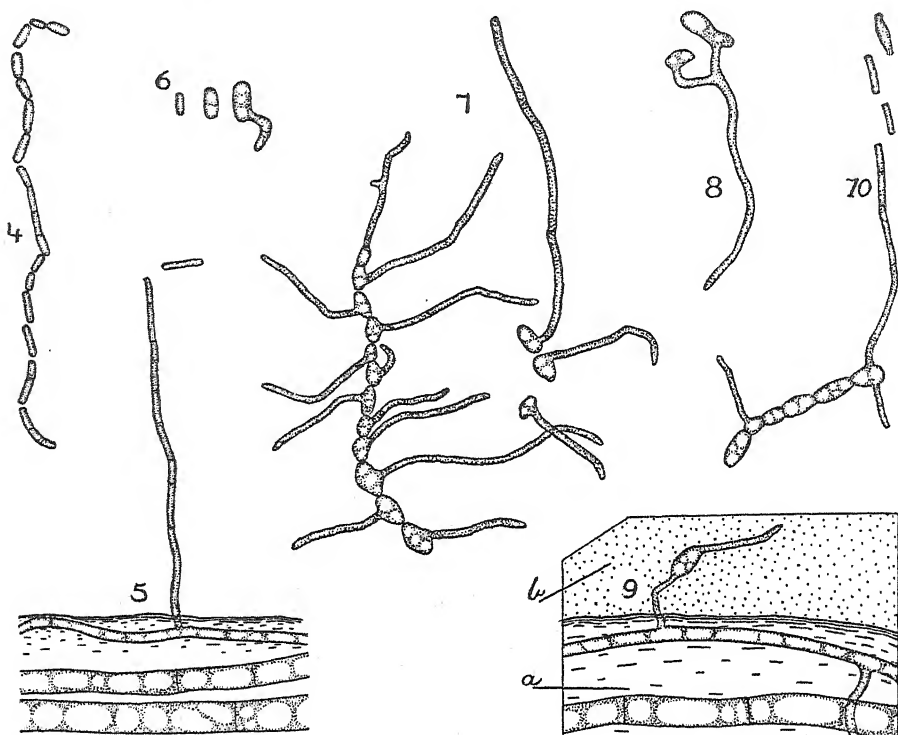
A mycelium which is about to produce oidia gives rise to a number of relatively slender aerial hyphae which grow out perpendicularly from the surface of the culture medium (Text-fig. 5). Then each hypha, beginning at its apex and proceeding downwards, breaks up into a series of short cylindrical cells which become the oidia (Text-fig. 4). These oidia, when fully formed, are loosely attached to one another and a chain of oidia may be readily detached from the mycelium by a puff of air blown from the mouth. The oidia adhere to any object which is brought into contact with them. Those shown in Plate XIX, Fig. 4, adhered to a glass slide which was lowered gently on to the surface of a mycelium.

The oidia of *A. stercorarius* germinate readily in water, dung, dung agar, or air saturated with water vapour, and in all these media germination begins within twelve hours after the oidia have been sown.

On germinating, an oidium first swells up to about eight times its original volume and, in so doing, becomes barrel-shaped and vacuolated (Text-fig. 6). Then it puts out either one or two germ-tubes from its sides or ends (Text-figs. 6-10). In a nutrient solution the germ-tubes soon develop into a branched and septate mycelium which, after growing for two or three days, gives rise to a new set of aerial hyphae which break

up in their turn into oidia. Sometimes, as shown in Text-fig. 10, the germ-tube itself breaks up into oidia.

The germ-tube of an oidium readily fuses with any other hypha with



TEXT-FIGS. 4-10. *Ascobolus stercorarius*. $\times 352$. 4. Oidia which developed as a chain, now detached from the mycelium. 5. A chain of oidia in course of formation from an aerial hypha projecting from a mycelium growing in dung agar (one oidium shown detached). 6. Three stages in the germination of an oidium, showing swelling and vacuolization. 7. Parts of two chains of oidia in which the oidia have germinated. The germ-tubes are already much elongated. 8. Two oidia have germinated and the germ-tubes have fused. 9. The edge of a hanging drop of dung agar, *a*, containing a mycelium. Outside the drop in a film of water, *b*, on the cover-glass is an oidium which developed on the mycelium and became isolated from it. This oidium has produced two germ-tubes one of which has fused with the parent mycelium. 10. A chain of six oidia which have swollen and are germinating. The end oidium on the right has developed two germ-tubes, one of which is breaking up into oidia.

which it comes into contact (Text-fig. 9). Thus such a germ-tube has been observed to fuse with the germ-tube of another oidium of the same or of opposite sex and with a hypha of a mycelium derived from an ascospore of the same or of opposite sex.

The significance of hyphal fusions in the mycelia of the Higher Fungi has recently been discussed at length by Buller (5). Where two mycelia derived from two ascospores or two oidia of opposite sex fuse together, sexual co-operation is made possible; and where many mycelia derived from ascospores or oidia of both sexes combine to form a compound

mycelium, the individual mycelia making up the compound mycelium are able to co-operate in the formation of an apothecium by supplying it with the materials required for its growth.

	1	9	6	8
1	—	—	+	+
9	—	—	+	+
6	+	+	—	—
8	+	+	—	—

TABLE III. *Ascobolus stercorarius*. All possible pairings between four masses of oidia, each mass derived from a mycelium of ascoporous origin.

To determine the sexual nature of the oidia of *A. stercorarius*, oidia produced on four mycelia of ascoporous origin and known sex were paired in all possible ways on the culture medium. The four mycelia were Nos. 1, 6, 8, and 9 of Table I, Nos. 1 and 9 being of one sex and Nos. 6 and 8 of the other and opposite sex. Each pair was established in the first instance by inoculating a hanging drop of dung agar with oidia from two of the four mycelia. The two inocula were placed on opposite sides of the drop. Each inoculum consisted of a mass of oidia, and each mass was obtained by drawing a sterilized needle through the aerial chains of oidia projecting above the surface of the mycelium which had produced them. The oidia which had been touched adhered to the surface of the needle. When the needle was brought into contact with the hanging drop of dung agar, the oidia were transferred to the drop. As soon as the two inocula had been deposited in a hanging drop, they were examined microscopically, when it was found that they consisted of oidia only. This was to be expected, as the aerial hyphae of the mycelium of *A. stercorarius* is made up entirely of chains of oidia.

Two days after the oidia had been paired, it could be seen with the microscope that in every hanging drop of dung agar the mycelia derived from the two masses of oidia had met and had fused so as to form a netted compound mycelium. Each compound mycelium was then transferred to sterilized horse dung in a wide-mouthed bottle. These mycelia grew well. Some produced fruit-bodies within a week of transference, while others did not and remained sterile for more than six weeks.

The results of pairing the oidia are embodied in Table III where, as before, a (+) sign indicates that the combination produced apothecia and a (—) sign that it did not. The original table of results was re-arranged so as to bring like oidial mycelia together.

An inspection of Table III shows that the oidia Nos. 1 and 9 are of one sex and the oidia Nos. 6 and 8 are of the other and opposite sex.

A comparison of Tables I and III shows that the oidia of the ascosporous mycelia Nos. 1, 6, 8, and 9 are of the same sex as the mycelia upon which they have been produced. We may conclude that all the oidia borne on any one mycelium derived from an ascospore are of one and the same sex, the sex of the ascospore.

V. MITES AS AGENTS IN TRANSFERRING OIDIA.

It was by accident that my attention was first drawn to the possibility of mites being agents in the transference of the oidia of *A. stercorarius*. Ten Petri dishes containing dung-agar, in each of which was growing a mycelium derived from a single ascospore, were placed in a pile one above another on a glass plate, and the pile of dishes was covered with a bell-jar. The plate and bell-jar had been swabbed with carbolic acid solution, and they and the dishes which they enclosed were set on a laboratory table and handled as little as possible, to prevent contamination. The ten mycelia were Nos. 11–20 of Table II, and they were being kept as stock cultures after the results embodied in Table II had been obtained. It was expected that they would remain sterile indefinitely. Twenty-eight days after the Petri dishes had been inoculated and enclosed under the bell-jar, it was observed that apothecia were developing in the dishes. The first dish to fruit was at the bottom of the pile, the second to fruit the next highest, and so on upward through the pile; and, finally, all the dishes fruited except the tenth and highest one.

An examination of the pile of ten Petri dishes which were fruiting in succession revealed the fact that the dishes had become invaded by mites (Acarineae).¹ These had come from an old unsterilized horse-dung culture and had crawled first over the table, then between the bell-jar and the plate on which the bell-jar stood, then through the space between each Petri dish and its cover and, finally, from one dish to another.

There was strong reason to suppose that the fruiting of the dishes in the pile of ten from below upwards was due to the mites having transferred oidia from one dish to another. The mites naturally crawled into the lower Petri dishes first and progressively ascended the pile.

After the observation just recorded had been made, a series of experiments was set up with a view to investigating under controlled conditions the transference of oidia by mites and the effect of the oidia so transferred on mycelia of opposite sex.

¹ Unfortunately, up to the present, it has not been possible to identify the species with which my investigations have been concerned. A mite known as *Tyroglyphus mycophagus*, which does damage to cultivated mushrooms, was described by Megnin in 1876. Possibly the Winnipeg species is related to it.

At the time when the investigation on mites and oidia was begun, Brodie (4) in the same laboratory was completing his experimental inquiry into the relation of flies with the oidia of *Coprinus lagopus*, and it was found convenient to make use of the apparatus that he had devised for his own work.

Twenty-two glass tubes, each 4 in. long and 1 in. wide, were partially filled with horse dung, plugged with cotton wool, and sterilized. Eleven of the tubes were then inoculated with mycelium No. 8 of Table I and the other eleven tubes with mycelium No. 9 of Table I. These mycelia were known to be of opposite sex. After a few days, when the mycelia had spread somewhat over the surface of the horse dung in each tube, a number of mites, which had been bred on horse dung, were introduced by means of a capillary glass tube into seven tubes containing mycelium No. 8 and into seven tubes containing mycelium No. 9. Four tubes of mycelium No. 8 and four tubes of mycelium No. 9 were thus left without mites, and these tubes served as controls. The eight control tubes were kept under conditions where mites could not possibly find access to them.

The seven tubes of mycelium No. 8 containing mites and the seven tubes of mycelium No. 9 containing mites were then combined into seven pairs. Each pair was made by taking one tube containing mycelium No. 8 and one tube containing mycelium No. 9, removing the plugs, and setting the tubes mouth to mouth, as shown in Pl. XIX, Fig. 5 (right). The two tubes of each pair were attached to one another by winding adhesive paper several times around their adjoined mouths, and then melted paraffin wax was poured over the paper so as to fill up any possible crevices. The four tubes of mycelium No. 8 without mites and the four tubes of mycelium No. 9 without mites were similarly paired and bound together. Care was taken to keep all the pairs of tubes from the first in a horizontal position, and thus to prevent any oidia from falling from one end of a pair of tubes to the other end.

By the end of fourteen days after the seven experimental pairs of tubes (with mites) and the four control pairs of tubes (without mites) had been set up, it was observed that apothecia had appeared on the dung at one end or at both ends of each of the seven experimental pairs of tubes, but that no apothecia had appeared on the dung in any of the four control pairs of tubes.

In none of the pairs of experimental tubes was it possible for the oidia to fall from one end to the other, and in none of them did the two mycelia creep along the surface of the glass and so come into contact with one another. The fact that apothecia were produced in all the experimental pairs of tubes and in none of the control pairs affords strong evidence that: (1) the mites acted as carriers of oidia from one mycelium to the other; (2) that the oidia of one sex when deposited by the mites on or

near a mycelium of opposite sex germinated; and (3) that the oidial germ-tube or mycelium so produced fused with the mycelium to which the oidia had been brought, and thus caused it to develop apothecia.

In two of the experimental pairs of tubes (with mites) apothecia appeared on both of the mycelia, and in the other five on one mycelium only. The non-production of apothecia by one of the two mycelia in a pair of tubes may have been due to the failure of the mites to deposit oidia on or near the mycelium under conditions of moisture suitable for germination. After an experimental pair of tubes has been set up, there are only a few days in which the mites can transfer oidia from one mycelium to another so as to enable these mycelia to produce fruit-bodies; for, in less than a week after the establishment of a pair of tubes, the mites may have fed upon and injured each mycelium to such an extent that, by this time, it has become hollowed out into a spongy mass or has been entirely destroyed.

Mites are very frequently associated with dung at Winnipeg, for they often appear in the laboratory on old horse-dung cultures. It may well be that, under natural conditions, they sometimes or often play a part in transferring oidia of *Ascoboli* and of other fungi from mycelia of one sex to mycelia of the opposite sex, thus aiding reproduction in the fungi concerned.

VI. THE EFFECT OF SOWING GERMINATING OIDIA NEAR AN ASCOSPOROUS MYCELIUM OF OPPOSITE SEX.

Mycelia Nos. 8 and 9 of Table I are of opposite sex. These two mycelia, each derived from an ascospore, were grown on dung in bottles. Two bottles contained mycelium No. 8 and two No. 9. Two hanging drops of dung agar were prepared. One of them was inoculated with oidia of mycelium No. 8 and the other with oidia from mycelium No. 9. In the course of a few hours the oidia in each of the drops began to germinate. The germinating oidia of mycelium No. 8, together with the drop of dung agar in which they had been sown, were then transferred to one of the bottles containing mycelium No. 9 and were set down beside the mycelium on the dung. The other bottle, containing mycelium No. 9, was kept as a control. Similarly, the germinating oidia of mycelium No. 9 were transferred to one of the bottles containing mycelium No. 8, and the other bottle containing mycelium No. 8 was kept as a control.

In the two experimental bottles apothecia appeared on the mycelia within a week after these mycelia had been inoculated with oidia, while in the two control bottles no apothecia appeared within six weeks, at the end of which time the experiment was discontinued.

One other experiment of the kind just described was made. Germinating oidia of mycelium No. 6 of Table I (of one sex) were added at

the periphery of mycelium No. 1 (of opposite sex), with the result that apothecia were formed in the bottle within a week, while the mycelium No. 1 in the control bottle (no oidia added) remained sterile.

The results of the series of experiments just recorded show that a mycelium derived from an oidium of one sex can interact with a mycelium derived from an ascospore of opposite sex, and thus can take part in the sexual process which culminates in the production of apothecia.

VII. FLIES AS AGENTS IN TRANSFERRING OIDIA.

The work of Brodie on the relation of flies and the oidia of *C. lagopus* has been referred to in the Introduction. *A. stercorarius*, like *C. lagopus*, is a coprophilous species, and it therefore seemed possible that flies might disperse its oidia. Experiments were therefore made to find out whether or not flies might be: (1) agents for the dispersal of *A. stercorarius* by transferring oidia from a mycelium to fresh dung; and (2) agents for indirectly initiating the sexual process by transferring oidia from a mycelium of one sex to a mycelium of opposite sex.

(1) *The transference of oidia to dung.* Some fruit flies, *Drosophila melanogaster*, bred on banana pulp, were kept in a bottle without food for twenty-four hours, and were then transferred to a Petri dish in which the mycelium of *A. stercorarius* was growing on horse dung. Projecting from the mycelium in the substratum were numerous chains of oidia, and to some of these were attached drops of a clear liquid which had been excreted. The flies were observed sucking up some of these drops, although they did not seem to be especially attracted by them. On the whole, the flies appeared to dislike walking among the projecting chains of oidia, and they soon passed from the mycelium to the free surface of the dung where they vigorously rubbed their legs. Perhaps they were attempting to free themselves from the oidia which must have been sticking to them.

After the flies had walked over the mycelium in the Petri dish, they were allowed to escape into other Petri dishes containing sterilized horse dung. One fly was imprisoned in each of three such dishes. In each dish the fly crawled once or twice over the surface of the dung. As soon as this had happened, the Petri dish was opened for a moment and the fly was allowed to escape.

Three or four days after the three flies had been removed from the three Petri dishes a vigorous mycelium appeared on the dung where the flies had crawled. The mycelium in each dish resembled that of *A. stercorarius*. On the assumption that the new mycelia had sprung from the original mycelium through oidia borne and deposited by the flies, some oidia of a sex opposite to that of the original mycelium were added to the

dung in one of the three Petri dishes containing the new mycelia. A few days afterwards, the mycelium to which the oidia had been added developed apothecia, whereas the other two mycelia remained sterile. This result is exactly what would be expected on the assumption that the three new mycelia had developed from oidia derived from the original mycelium.

The experiments just recorded show that flies are able to carry oidia from a mycelium to the surface of fresh dung, and thus act as agents for the dispersal of oidia.

(2) *The transference of oidia from one mycelium to another of opposite sex.* Six glass tubes, 4 in. long and 1 in. wide (like those already used for the experiments with mites), were partially filled with horse dung, plugged with cotton wool, and sterilized.

The mycelia employed were Nos. 1, 6, 8, and 9 of Table I. No. 1 and No. 6 are of opposite sex; and No. 8 and No. 9 are of opposite sex.

The six tubes of sterilized dung were inoculated as follows: two with mycelium No. 1, two with mycelium No. 6, one with mycelium No. 8, and one with mycelium No. 9.

As soon as the mycelia had begun to grow well in the tubes, two flies were introduced into one of the No. 1 tubes, one of the No. 6 tubes, the No. 8 tube, and the No. 9 tube. The other No. 1 tube and the other No. 6 tube were left uninoculated, so that they might be used as controls.

The four tubes containing flies were paired (after removal of their plugs) mouth to mouth (Pl. XIX, Fig. 5, left) as follows: No. 1 with No. 6, and No. 8 with No. 9. The two tubes without flies, No. 1 and No. 6, were also set mouth to mouth. The two tubes of each pair were fastened together by means of a cylinder of cardboard slipped about their adjoined mouths, and the space between the cardboard and the glass was filled with cotton wool.

Within a week after adding the flies to the tubes and combining the tubes mouth to mouth in pairs, the experimental tube-pairs Nos. 1 and 6 and Nos. 8 and 9 containing flies produced a large number of apothecia (Pl. XIX, Fig. 5, left), while the control tube-pair Nos. 1 and 6, to which no flies had been added, remained sterile. The sterility of the two mycelia in the control tube-pair continued for two weeks, at the end of which time all the cultures were discarded.

The series of experiments just described indicates: (1) that flies can transport the oidia of *A. stercorarius* from one mycelium to another of opposite sex; (2) that the oidia can then germinate; and (3) that the oidial germ-tubes or mycelia can fuse with the mycelium to which the oidia have been brought, and so initiate the sexual process resulting in the production of apothecia.

VIII. WIND AS A FACTOR IN DISPERSING OIDIA.

Three Petri dishes containing sterilized dung were inoculated with bits of a mycelium derived from an ascospore. Each of the three new mycelia thus obtained grew well and soon produced an abundance of oidia. Fresh dung was placed in three more Petri dishes, pressed down tightly, and sterilized. Next, the covers of all the six Petri dishes were removed, and each Petri dish containing a mycelium was covered with an inverted dish containing sterilized dung only. An apparatus for giving a blast of air was made by attaching a piece of rubber tubing to a glass tube drawn out at the free end until this was about 1 mm. in diameter. The upper member of each pair of dishes was then lifted slightly, and the end of the glass tube was inserted into the opening of the chamber. The free end of the rubber tubing was placed in the mouth, and a blast of air was then blown at the mycelium on the dung in the lower dish with a force just sufficient to cause the chains of oidia to bend before it. The two dishes of each pair were then separated and covered with their original lids.

Three or four days after the mycelia had been blown upon, a rich growth of mycelium resembling that of *A. stercorarius* appeared on the dung of each dish which had been used as a cover in the three pairs of dishes.

The results of the series of experiments just described seem to indicate that oidia can be blown off the surface of a mycelium into the air, that they can settle on dung if they happen to come into contact with it, and that they can then germinate.

If oidia of *A. stercorarius* can be blown away from a mycelium in the laboratory, it may well be that oidia of this species may be blown away from a mycelium growing on dung in pastures. Conceivably oidia carried off by the wind might settle on freshly deposited dung and germinate there, and the mycelium so produced might fuse with a mycelium of opposite sex, and so initiate the development of apothecia.

IX. SUMMARY.

1. In *A. stercorarius* heating the spores to a temperature of 37.5° C. increases the number of spores which germinate.
2. Horse dung is a much better culture medium than dung-agar for the production of apothecia by mated mycelia.
3. The ascospores are unisexual and the fungus is heterothallic. The ascospores and the mycelia which they produce are sexually of two kinds. One kind may be regarded as (+) and the other as (-). When two (+) mycelia or two (-) mycelia are paired, the pairs remain sterile indefinitely, whereas, when a (+) mycelium and a (-) mycelium are paired, large numbers of apothecia are soon formed.

4. The mycelium derived from an ascospore of *A. stercorarius* produces chains of oidia projecting into the air above the substratum. The oidia germinate freely in water, horse-dung agar, and horse dung. The mycelium derived from an oidium again produces oidia.

5. The oidia are unisexual. A (+) mycelium of ascosporous origin produces (+) oidia and a (−) mycelium of ascosporous origin produces (−) oidia. When two (+) mycelia or two (−) mycelia derived from oidia are paired, the pairs remain sterile indefinitely; but, when a mycelium derived from (+) oidia is paired with a mycelium derived from (−) oidia, large numbers of apothecia are soon formed.

6. The mites which infest horse dung feed upon the mycelium of *A. stercorarius*.

7. When mites are allowed to crawl from a mycelium of one sex bearing oidia to another mycelium of opposite sex, apothecia are produced in the culture visited by the mites. It is inferred that, in such an experiment, the mites carry oidia from the first mycelium to the second, that the oidia germinate, that the new mycelium fuses with the old one, and that thus the sexual process resulting in the production of apothecia is initiated.

8. When germinating oidia of one sex are transferred by hand to a dung culture containing an ascosporous mycelium of opposite sex, apothecia soon appear in the culture.

9. Flies, like mites, are able to carry oidia away from mycelia and to deposit them elsewhere. When oidia are carried by flies on to sterilized horse dung, the oidia germinate there and give rise to new mycelia which develop more oidia. When flies are allowed to pass from a mycelium of one sex bearing oidia to another mycelium of opposite sex, apothecia are produced in the culture visited by the flies. It is inferred that, in such an experiment, the flies carry oidia from the first mycelium to the second, that the oidia germinate, that the new mycelium fuses with the old one, and that thus the sexual process resulting in apothecia is initiated.

10. It seems probable that, under natural conditions in the open, dung flies and mites are agents in transferring oidia from one mycelium to another.

11. A blast of air may detach oidia from the mycelium on which they are produced. Possibly, the wind, also, is an agent in transporting oidia from one mycelium to another.

The investigation was carried out in the Department of Botany of the University of Manitoba during my tenure of the Hudson's Bay Company Research Fellowship, 1929–30. It gives me great pleasure to acknowledge my indebtedness to the kindness of Professor A. H. Reginald Buller, whose suggestions and criticisms during the progress of the work have been invaluable.

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EXPLANATION OF PLATE XIX.

Illustrating Dr. E. Silver Dowding's paper on *Ascobolus stercorarius*.

All figures are those of *Ascobolus stercorarius*.

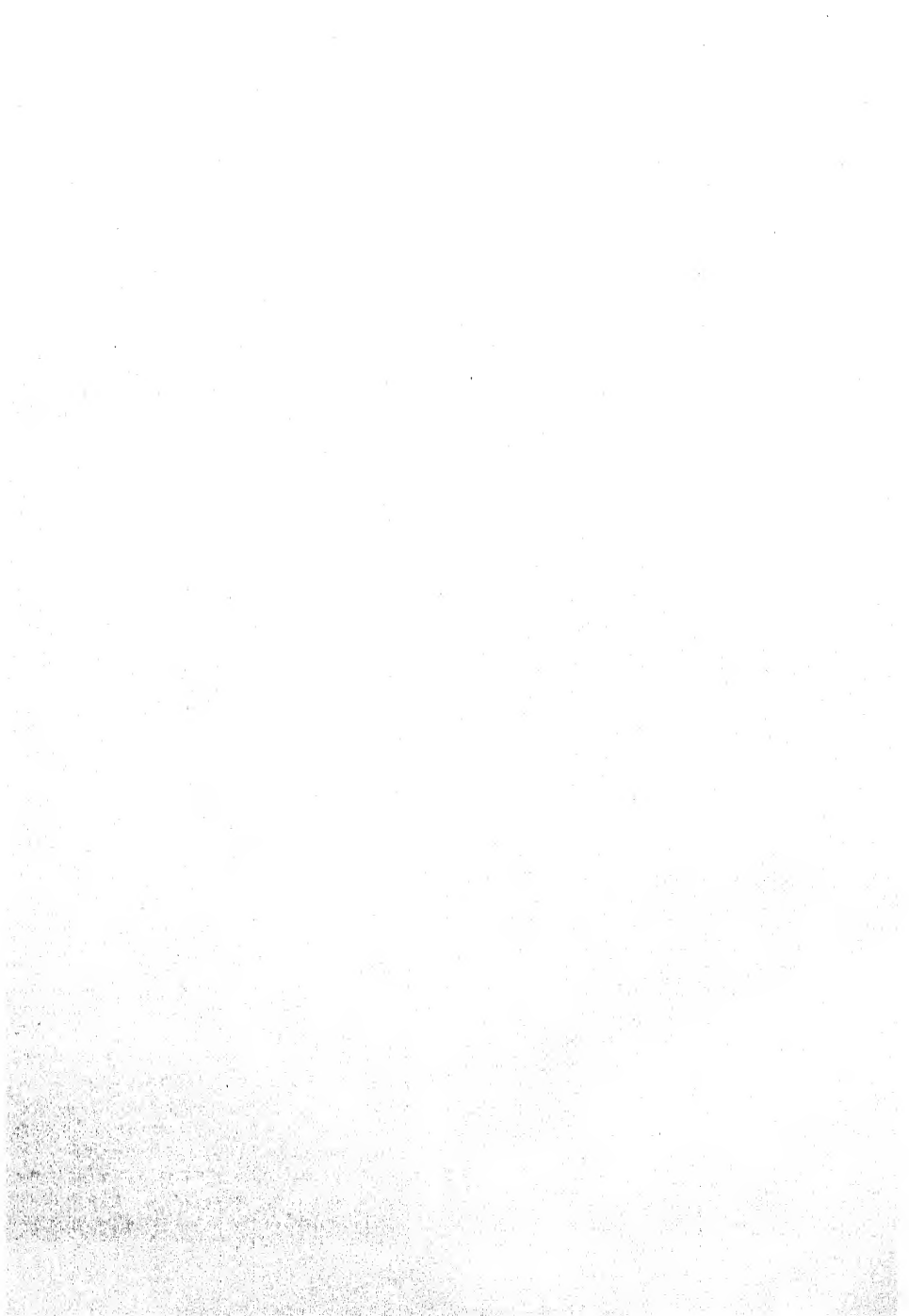
Fig. 1. A mycelium which originated from a single ascospore is growing on sterilized horse dung. This culture was kept for four months and remained perfectly sterile. Natural size.

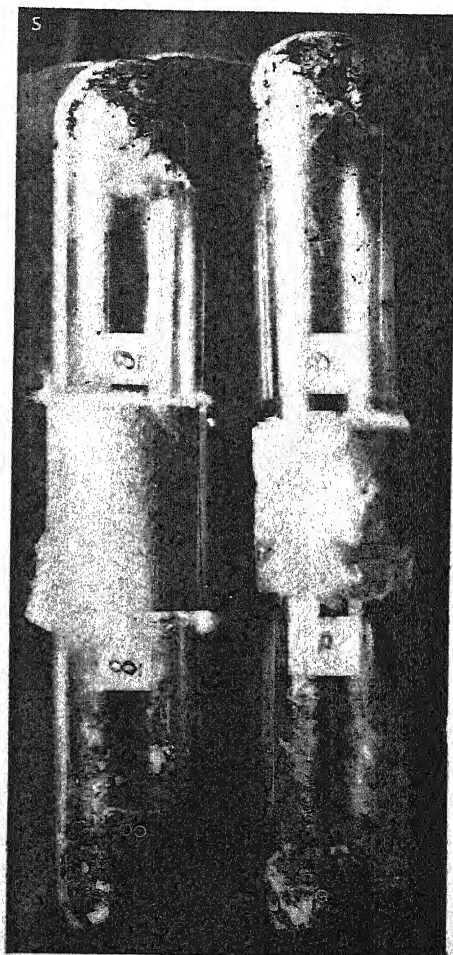
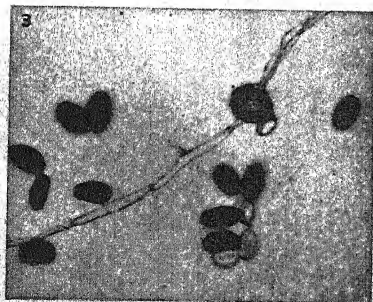
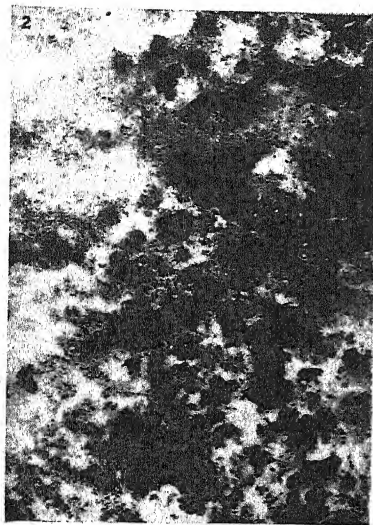
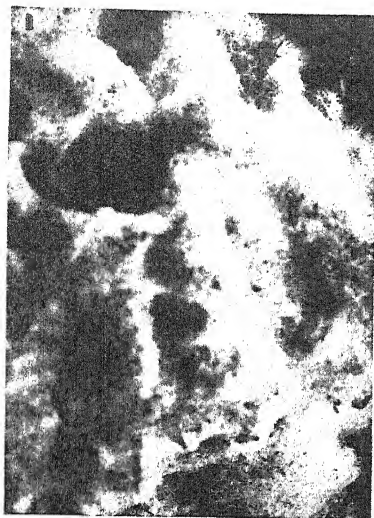
Fig. 2. A mycelium which originated from the mating of two mycelia derived from ascospores of opposite sex. The culture is two weeks old, and it has produced a number of apothecia. The apothecia to the left of the centre of the photograph are expanding; those at the top and at the bottom-right appear black and are fully expanded. Natural size.

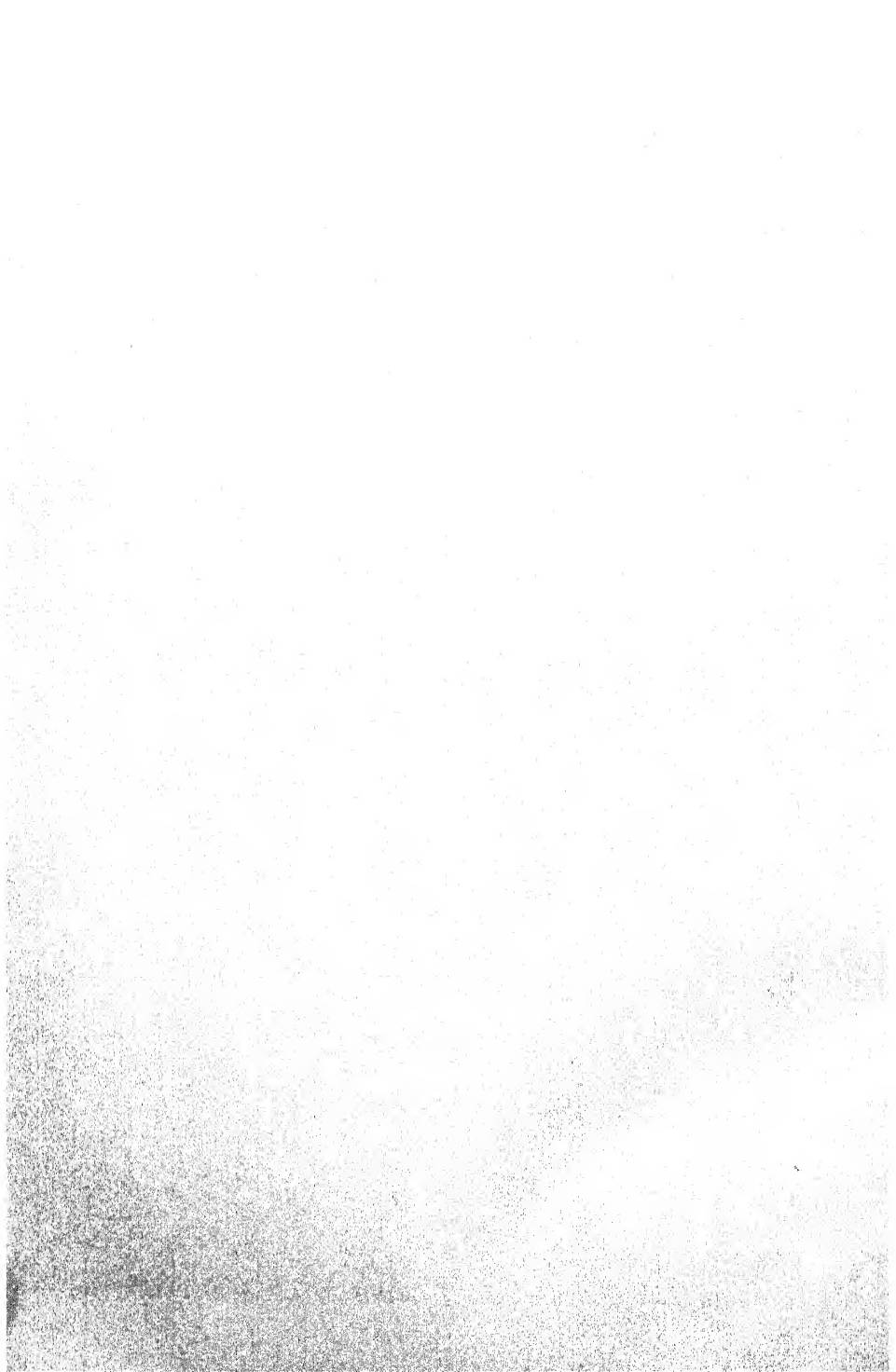
Fig. 3. A photomicrograph of a spore-deposit in which can be distinguished four small hyaline ascospores and several larger dark-coloured ascospores. One of the latter has swollen and has germinated. Magnification, about 230.

Fig. 4. A photomicrograph of a deposit of oidia. A glass slide was dabbed on to the aerial mycelium of a culture and the oidia adhered to the glass, some singly and others still in the chains in which they were formed. Magnification, about 230.

Fig. 5. Two tube-pairs, the one on the right at the end of an experiment made with mites, the one on the left at the end of an experiment made with flies. Each tube-pair consists of two glass tubes placed mouth to mouth, the combined mouths being sealed. In the right tube-pair the seal is adhesive tape and paraffin wax; in the left tube-pair the seal is a cardboard cylinder and cotton wool. At the two ends of each tube-pair is a mass of horse dung which was inoculated with a unisexual mycelium derived from a single ascospore. In a tube-pair, one mycelium was originally of one sex and the other mycelium of opposite sex (Nos. 8 and 9 of Table I). Mites were enclosed in the right tube-pair and flies in the left tube-pair. In each of these experiments the mycelia at both ends of each tube-pair developed fruit bodies at the end of a week from the establishment of the tube-pair. The position of some of the apothecia is indicated by surrounding black or white circles. It is inferred that the development of the apothecia at the ends of each tube-pair was due to the transfer by the mites or flies of oidia from one mycelium to the other, the germination of the oidia so transported, and the interaction of the mycelium of oidial origin with the mycelium of ascospore origin. Reduced to two-thirds the natural size.







Species Hybrids in *Aquilegia*.

BY

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AND

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With four Figures in the Text.

FROM a morphological point of view most studies of species hybrids have been relatively unproductive. Mendelian analysis, though it has been of great assistance in the investigation of varietal differences, has been possible in only a few instances—East (4), Chittenden (1). Even in these favoured cases it has done little more than demonstrate that certain factors probably behave much the same way in hybrids as they do in the parent species.

In the following preliminary investigation of species crosses in *Aquilegia*, an attempt has been made to devise methods of morphological analysis which will on the one hand be useful in codifying the data from crosses between species, and which, on the other hand, will yield objective evidence as to the probable course of evolutionary development. This preliminary account is published to draw the attention of other workers to the problem in the hope that further methods may be developed.

For a general study of interspecific hybrids the genus *Aquilegia* is excellent material. Great morphological diversity between species is accompanied by unusual interfertility. The genus numbers roughly eighty to one hundred species, native to Europe, Asia, and North America. They exhibit remarkable morphological and ecological diversity, and can be grouped into at least five different sections. In spite of this diversity, every species cross which has been attempted has produced a first generation hybrid, and all of these have been at least partially fertile.

Morphologically the genus is particularly interesting by reason of its distinctive petals, which are sometimes interpreted as modified honey

glands (6). Fig. 1 represents the petals of eight species drawn to scale. As shown in the figure, they are developed anteriorly into a broad blade or 'lamina', and prolonged below into a more or less narrow spur with the actual

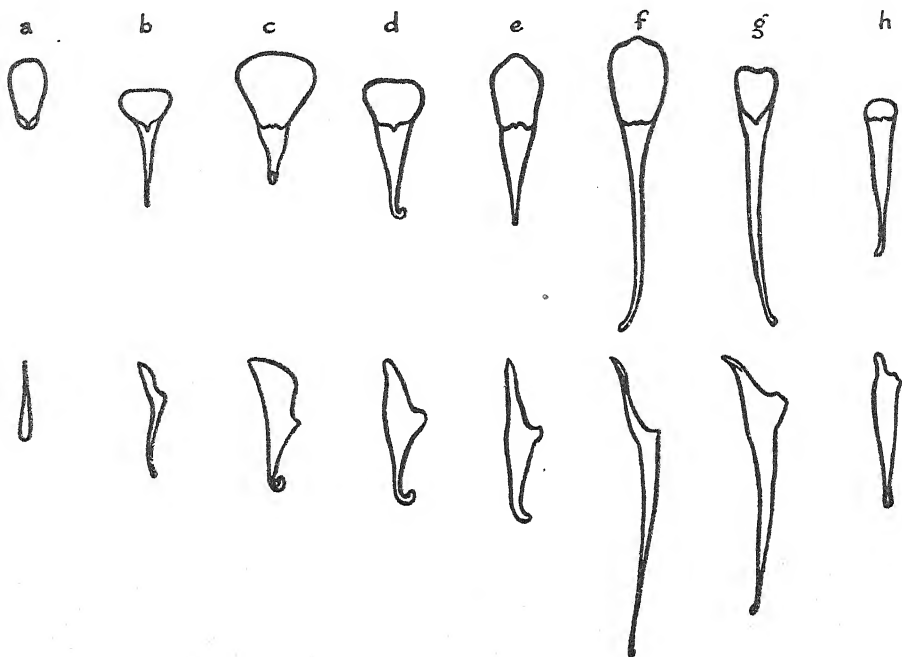


FIG. 1. Petals of various species of *Aquilegia*, in full face and in profile. (a) *A. ecalcarata* Maxim. (b) *A. viridiflora*, Pall. (c) *A. fragrans*, Benth. (d) *A. vulgaris*, Linn. (e) *A. pyrenaica*, DC. (f) *A. caerulea*, James. (g) *A. Skinneri*, Hook. (h) *A. canadensis*, Linn. Natural size.

gland at its apex. In the widespread section *Cyrtoplectrae* (*A. vulgaris*, d; and *A. pyrenaica*, e), which includes species in Europe, Asia, and America, the lamina and spur are about equally developed, and the latter is more or less incurved. Related to this section are several curious Asiatic species (represented in the figure by *A. viridiflora*, b; and *A. fragrans*, c) in which the lamina is much wider than long. In the living flower the wide laminae overlap and produce a peculiar cuplike effect. The American sections, *Rhodanthe* and *Macroplectrae*, are both characterized by long and narrow spurs. In the *Macroplectrae* (*A. caerulea*, f) the lamina is also well developed. In one member of this group, *A. longissima*, the spurs sometimes reach a length of 15 centimetres. In the section *Rhodanthe*, represented in the figure by *A. canadensis* (h) and *A. Skinneri* (g), the spurs are well developed and the laminae are short.

In the Chinese species, *A. ecalcarata*, Maxim. (Fig. 1, a), and a few other related forms, the spur is represented by a small pouchlike gland.

There are good reasons for believing that this represents the ancestral condition of the genus rather than a 'reduction'. In the first place it is similar to the honey glands at the bases of the petals in many of the related genera of the Ranunculaceae. In the second place, *A. ecalcarata* differs from the remaining species of the genus in several other characters, such as the shape of its fruit, the colour of its flowers, and its general aspect. In all of these characters it reminds one of certain genera closely related to *Aquilegia*, such as *Isopyrum* or *Anemone*. Drummond and Hutchinson (3) even transferred it to Makino's genus *Semi-aquilegia*, concerning which they wrote: '*Semi-aquilegia*, with saccate petals, the only remaining link showing the origin of the remarkable genus *Aquilegia*, which as evidenced by the great variability of its species and its wide range, is probably still in a state of flux.'

Be that as it may,¹ we undoubtedly do possess in the spur of *Aquilegia* a series of forms showing various degrees of similarity, which might easily be arranged in a phylogenetic sequence. But in *Aquilegia* such a speculative arrangement can be treated experimentally, for the various members of the series are interfertile and can be crossed one with another. Not only that, but in *ecalcarata* we even possess 'a living fossil', similar to the ancestral type in many respects, yet capable of producing fertile hybrids with its more highly elaborated sister species.

Cytologically, *Aquilegia* is not very favourable material. The chromosomes are very small; the pollen mother-cells are not easy to fix. The following numbers have previously been reported :

	<i>n.</i>	<i>2n.</i>	Reported by
<i>A. atropurpurea</i>		14	Langlet
<i>A. chrysantha</i>	7		Scalinska
<i>A. haylodgensis</i> , Hort.		14	Langlet
<i>A. vulgaris</i>	7		Winge
<i>A. vulgaris parviflora</i>		14	Langlet
<i>A. vulgaris</i> × <i>chrysantha</i>	7		Skalinska

The following root-tip counts were made :

<i>A. flabellata</i> , Hort.	14
<i>A. chrysantha</i> , Gray (New Mexico)	16
<i>A. chrysantha</i> , Gray (Arizona).	16
<i>A. vulgaris</i> , var. 'China Blue'	16 ²

The chromosome numbers reported above indicate no gross chromo-

¹ See, for instance, Ulbrich (8).

² The *flabellata* was of commercial origin. The specimens of *chrysantha* were grown from seed from wild stands. 'China Blue' is the laboratory name of a variety which turned up among the stocks originally got together at the John Innes Institution from various sources and handed over to the junior author in 1927. Its origin is unknown to us; it clearly belongs to *A. vulgaris*, L. and it breeds true. It is slow growing, about half the height of typical *vulgaris* but with flowers of the same size. The flowers are of a light-blue colour and tinged red in the bud. This very distinct variety has clearly originated from type *vulgaris* by some form of cytological aberration.

somal differences in the genus. *Aquilegia* is, therefore, one of those genera like *Ribes*, in which sizes, shapes, and numbers of the chromosomes remain relatively constant in spite of great external differentiation between the species.

The work reported below was begun independently by the senior and junior authors at the Missouri Botanical Garden and the John Innes Horticultural Institution respectively. In each case material of as many species as possible was obtained from seedsmen and botanic gardens, and a number of crosses were made. Experience has proved that practically all of this material is unreliable for precise work, and the study is being repeated with authentic material. The following crosses, however, were considered worthy of a preliminary examination, and it is with the results obtained from them that the present paper is concerned.

- A. vulgaris compacta* × *A. formosa* (1).
- A. caerulea* × *A. vulgaris* (2).
- A. ecalcarata* × *A. chrysantha* (3).
- A. ecalcarata* × *A. vulgaris compacta* (4).
- A. chrysantha* × *A. vulgaris* 'China Blue' (5).
- A. vulgaris* × *A. Jaeschkani* (6).
- A. vulgaris* × *A. chrysantha* (7).
- A. vulgaris* × *A. canadensis* (8).

Certain regularities of interspecific dominance were apparent even in this material. They can be roughly summarized by saying that all of the first generation hybrids were more like *Aquilegia vulgaris* (in the broad sense) than might have been expected. This was true even when *A. vulgaris* was not one of the parents. Similar results have been obtained by other investigators. Cockerell (2), for instance, crossed two American species, *A. chrysantha* and *A. desertorum*. As regards the hybrid he wrote: 'the form of the flowers departs from both parents in the direction of the *A. vulgaris* group'. In none of the cases in the literature, however, were enough different crosses attempted to suggest that the 'vulgaris' appearance of the F_1 was a general phenomenon. It may be mentioned, however, that it is a common experience of those who grow *Aquilegia* as a garden flower that the vast majority of seedlings from natural seed have the appearance of *vulgaris*.

Three of the most interesting crosses are illustrated in Fig. 2. The parents used were:

1. *A. ecalcarata*, Maxim. (with practically no spur).
2. Garden varieties of an American long-spurred species.
3. A garden variety of *A. vulgaris* (medium spurred).

In contrast to the great differences between the parents is the remark-

able similarity of the hybrids. Theoretically we might have expected half-sister hybrids to show half the divergence of their unlike parents. That is to say, if we call the three species A, B, and C, and the F_1 hybrid between

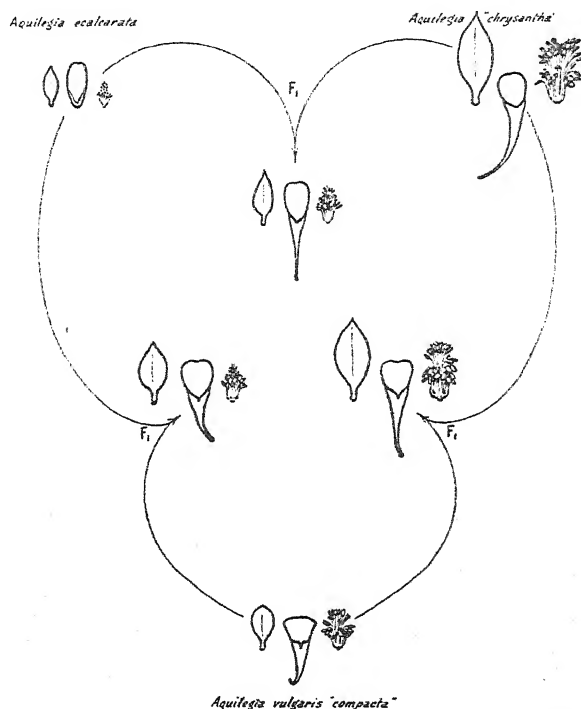


FIG. 2. Dissections of *A. ecalcarata*, *A. chrysantha* (garden form), and *A. vulgaris compacta* (garden form), with the F_1 hybrids between them. The hybrids are all very much alike, and resemble *vulgaris* much more than they do either of the other two species involved.

A and B is represented as AB, and that between A and C as AC, and so on, we might have expected the following relations to hold :

$$\frac{1}{2}(A-B) = AC-BC.$$

$$\frac{1}{2}(A-C) = AB-BC.$$

$$\frac{1}{2}(B-C) = AB-AC.$$

A mere glance at Fig. 2 is enough to show that this is clearly not the case. The three hybrids are very much like each other, in spite of the great differences between their parents. If we test the matter objectively, our opinion is adequately confirmed.

Not only are these three hybrids much more alike than we might reasonably have expected, but they are more like *A. vulgaris* than they are like any of the other species involved. This is equally true of the other crosses studied. An attempt was made to measure the resemblances more exactly,

and the results are presented in Figs. 3 and 4. Two characters were chosen for study, spur length and lamina length. In order to remove the effects of hybrid vigour, in so far as they cause changes in size, comparative ratios

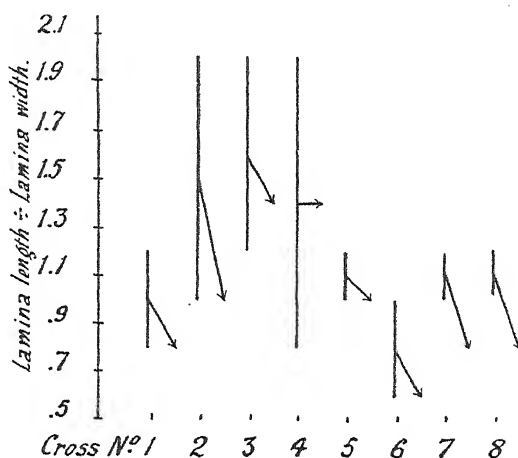


FIG. 3. Analysis of the ratio lamina length/lamina width in first generation hybrids. The vertical lines represent the difference between the parents in respect of this character. The arrow indicates the position of the F_1 hybrid on the same scale. 1. *A. vulgaris compacta* \times *A. formosa*. 2. *A. caerulea* \times *A. vulgaris*. 3. *A. ecalcarata* \times *A. chrysantha*. 4. *A. ecalcarata* \times *A. vulgaris compacta*. 5. *A. chrysantha* \times *A. vulgaris* 'China blue'. 6. *A. vulgaris* \times *A. Jaeschkeani*. 7. *A. vulgaris* \times *A. chrysantha*. 8. *A. vulgaris* \times *A. canadensis*.

were used. Since the width of the lamina at the orifice is a relatively constant character it was used as a basis for comparison, the actual ratios studied being:

1. Length of lamina/width of lamina.
2. Length of spur/width of lamina.

Figs. 3 and 4 represent for each of the eight crosses the position of the parent species on the two scales, the theoretical intermediate positions for the first generation hybrid, and its actual position (at the right).

The tables present in a more exact way what has already been stated in general terms. In every case where the hybrid is not at the theoretical midpoint between the parent species, the shift is in the direction of medium length spurs and wide laminae. In other words, in species crosses in *Aquilegia* there is a general dominance of medium length spurs over very long or very short spurs, and of wide laminae over long laminae. This is without any evident relation to the particular species used, and is irrespective of the direction in which the crosses are made.

The regularity of the phenomenon seems to be adequately established even by these preliminary studies. For its accurate interpretation further work with more authentic material will be necessary. Were it not for the results obtained with *A. ecalcarata* one might ascribe the phenomenon to

recombination, such, for instance, as is responsible for 'atavism' in crosses between breeds of domestic animals. But *A. ecalcarata*, though certainly more 'primitive' in its characters than *A. vulgaris*, is quite as recessive to *A. vulgaris* as are the 'advanced' American long-spurred species.

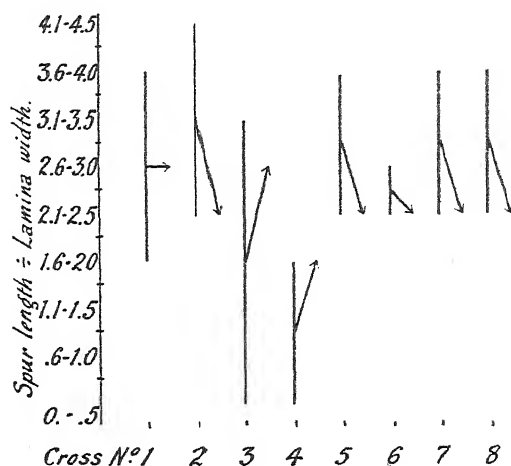


FIG. 4. Similar to Fig. 3, but giving the ratio spur length/lamina width.

It is not impossible that the whole problem is a more purely physiological one than might have been expected. It may be that the nuclear substances which produce medium spurs and wide laminae are more effective when diluted than those which produce long spurs and long laminae. In that case we are down to the physiological growth relationships which have limited in a broad way the directions of evolution within the genus.

The authors wish to thank Miss Irma Andersson-Kottö, who provided the nucleus of the material in 1927.

Part of the work reported upon in the above paper was performed while the senior author was a fellow of the National Research Council, to which due acknowledgement is made.

SUMMARY.

Preliminary studies indicate that species hybrids in the genus *Aquilegia* tend to resemble *A. vulgaris*, even where that species is not one of the parents.

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The Early Evolution of the Angiosperms.¹

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With nine Figures in the Text.

INTRODUCTION.

THE antiquity and early evolution of the angiosperms are still most obscure and debatable subjects, and very little real advance has yet been made in elucidating them. The recent review of the literature by Bancroft (2, p. 153) shows that our position is not materially different from that outlined by Scott in 1924 (29, p. 32, ch. ii).

In the present paper no further attempt is made to summarize the opinions of writers on the subject, but the author has tried to develop more fully the tentative suggestions made a few years ago (38, p. 22), and to put forward certain considerations which indicate the need for a careful re-examination of the flowering plants in the light of fossil evidence.

During the past few years the current ideas on the ancestry and interrelations of the flowering plants have been reviewed by several of the leading authorities on taxonomy, and noteworthy pronouncements have been made by Engler (9, p. 130), Wettstein (43, p. 515), Rendle (24, p. 29), and Hutchinson (14). This subject has also been summarized by Campbell (6, p. 479). The conclusion that emerges clearly from these discussions is that we are completely ignorant of the really primitive angiosperms. All the living forms are so highly evolved that they give no indication of the source from which they sprang; there is nothing to indicate the type of plant in which the angiospermous habit first arose, or even the nature of the structure from which the closed ovary originated. It seems as though fossils alone can supply the necessary clues to the problem, and though we may never find the remains of the direct ancestors of our flowering plants, it may be that indications of their probable mode of evolution will be obtained from the study of the Mesozoic floras. If only

¹ The substance of this paper was read to Section PB. of the Fifth International Botanical Congress, 1930.

[*Annals of Botany*, Vol. XLV, No. CLXXX, October, 1931.]

a few guiding ideas could be established by reference to fossil plants, much could doubtless be extracted from our wealth of detailed knowledge about the flowering plants, for it is clear that valuable information is still to be obtained from the comparative study of modern forms. The work on the gynaecium which is being carried on at Ithaca under the leadership of Professor Eames, and the work of Miss Saunders at Cambridge, is likely to lead to helpful results. The evolution of the angiospermous habit must be intimately associated with the structure of the carpel and, as will be shown later, accurate information¹ on this subject is still badly needed. It is hoped that the present communication will indicate other directions in which information should be sought among the flowering plants.

Pre-Cretaceous Angiosperms.

In spite of all the research into the fossil floras which has been accomplished in recent years, no pre-Cretaceous plants are yet known which can be regarded as undoubted ancestors of the flowering plants. We must remember, however, that almost all of our known floras are the remains of delta or marsh plants, and may not be truly representative of the vegetation of the time. Fragments of leaves such as Seward's *Phyllites* (30, p. 152 Pl. XI, Figs. 5 and 6) from the Stonesfield Slate, of wood such as Kräusel's newly described *Suevioxylon zonatum* (17, p. 250), and perhaps the leaf described by Salfeld (26) as *Ungeria solnhofensis* from the Solnhofen lithographic shale, undoubtedly suggest that flowering plants existed in Jurassic times, but they do not help us very much in our quest for ancestral forms.

When we examine the Upper Cretaceous angiosperms we find widely diverse types already evolved, widely spread in the world, and some with an organization which has changed but little since that time. The most reasonable deduction from the facts is that these plants had already undergone a long course of evolution, and that truly ancestral angiosperms must be sought at a much earlier period. I therefore accept the late Professor Engler's view (9, p. 138) that a well-differentiated group of angiosperms probably existed in pre-Cretaceous times.

Now if this postulated group existed in Triassic and Jurassic times we should naturally expect to find traces of it in the marsh floras, which in several cases have been so exhaustively studied. The question must then be asked, Are there indeed no traces of such plants? In trying to answer this question, we must remember that we are almost entirely ignorant of the actual characters of the early angiosperms. We have no proof of the correctness of Engler's ideas as to the characters of his postulated Protangiosperm group; the actual plants may have been very different from

¹ This view is admirably stated by Mrs. Arber (1), p. 12.

anything yet imagined or found to-day. Or, again, the marsh Protangiosperms of the Jurassic may have been archaic forms persisting in a semi-aquatic habitat. A third possibility is that the early angiosperms, like many other groups, were evolving along several different lines, and if marsh forms existed they may have been diverging from the series of more successful forms growing on dry ground.

In order to answer our question, the only way is to examine all the known megaphyllous seed plants of the early Mesozoic, and find whether they had any features in common with the modern angiosperms, or whether they suggest any evolutionary tendencies likely to lead to the development of structures characteristic of the flowering plants by the operation of factors which can be detected as influencing the differentiation of the angiosperms at a later date. Most botanists agree that modern flowering plants show tendencies towards a reduction in the number of spores formed in the flower, and also towards a fusion of the floral parts; so, when we encounter a Mesozoic reproductive structure, we must think what it would become if reduced in fertility and fused with other parts.

From this point of view the fossils attributed to the Cycadales, Bennettiales, and Pteridospermae should be carefully examined.

The Mesozoic Gymnosperms.

The Coniferales and Ginkgoales scarcely concern us. We know a good deal about the Bennettiales, which have occupied an important place in recent discussions. Our knowledge of the fossil Cycadales, which are widely different from the Bennettiales in their reproductive structures, is very meagre, and we have as yet little information about the pteridosperms, which undoubtedly persisted into the Mesozoic period. But we must remember that there were probably other groups living at the time whose reproductive structures are yet quite unknown to us. For example, we know nothing about types of plants to which the fronds called *Ctenis* and *Anthrophyopsis* belonged.

It is not necessary to deal with each group in detail, but certain points may be noticed.

(1) The leaves referable to all of these groups are generally compound. Very few examples of simple leaves, e.g. *Taeniopteris vittata* and *Nilssonia orientalis*, are known, and even these are closely related to compound forms, and occasionally show a divided lamina. It is, then, probable that the leaves of the earliest angiosperms were compound, and their sporophylls may have been compound also.

(2) The Mesozoic Cycadales have not been found to possess any features in which they resemble the angiosperms more closely than do the modern cycads. Although it is possible that both cycads and angiosperms

sprang from the same Palaeozoic stock, they must have diverged at an early period in their history.

(3) Although many authors have favoured the views of Arber and Parkin on the relation between the earliest angiosperms and the Bennettiales, and some have even suggested a direct connexion between these groups, it must be remembered that all the known Bennettiales differ markedly from angiosperms. The habit of the earlier types *Wielandiella* and *Williamsoniella* (35, p. 132, Text-figs. 5 and 20, p. 13) comes nearer to that of certain woody Dicotyledons (especially the Proteaceae, e.g. *Leucodendron argenteum*, where flower buds occur terminating the main shoot, and two or three stout branches are formed below them) than does that of the dwarf columnar Cycadeoideas of the Lower Cretaceous, but they are not very comparable to the more typical Dicotyledons. The Bennettialean leaves are very stereotyped in general form, with their pinnae produced from the upper side of the rachis. Reticulate venation appears as an exception in *Dictyozamites*, but this must be a case of evolution running along a course parallel to that seen in the ferns and pteridosperms, and probably to that of the angiosperms.

The ovulate strobili are remarkably constant throughout the group, and never show any approach to true angiospermy. The microsporophylls, with their transversely septate synangia, are never closely comparable with angiosperm stamens, and seem to have been diverging from the flowering plants in the course of their evolution. The important feature for comparison possessed by the Bennettiales is the formation of flowerlike structures, which were in some cases undoubtedly bisexual, and in others almost certainly unisexual (36, p. 109). Although this is almost the only point which the two groups have in common, it is of great interest and importance, especially when considered in conjunction with the Caytoniales.

The Caytoniales.

This group furnishes us with examples of plants which had attained angiospermy by the middle of the Mesozoic period, and which have therefore some claim to be regarded as protangiosperms, though they are in many respects more similar to the pteridosperms than to the flowering plants. Before considering their bearing on the problems at issue, something must be said about the evidence on which this group was reconstructed, and reference must be made to some criticisms which have been made upon my original paper (37).

A brief summary of our present knowledge of these fossils will now be given. The group named the Caytoniales was founded to include certain leaves, microsporophylls, and megasporophylls, which have never been found in organic connexion, and have therefore been given different generic

and specific names. Nothing is known of the stems which bore them, which were probably woody and had deciduous leaves. It is quite possible that we shall never succeed in finding specimens with the different parts in organic connexion.

The more characteristic features of the various parts are :

The leaves, for which we retain the name of *Sagenopteris*, are compound as are those of most of the known members of the Bennettitales, Cycadales, and pteridosperms. Usually two pairs of pinnae occur near the end of the petiole close together, but sometimes slightly separated. Occasionally palmate forms with three leaflets occur, as do simple forms with an orbicular lamina terminating the petiole. The venation is in the form of a simple reticulum. The cuticle resembles that of a mesophytic angiosperm, and the stomata are of the angiospermous type.

The microsporophylls had a pinnate form, the apex and lateral branches showing dichotomous divisions. Some lateral branches show repeated dichotomies, and were terminated by groups of anthers; the exact size and arrangement of the groups is yet unknown. Each anther had four longitudinal pollen sacs. The pollen grains had two wings symmetrically placed. These microsporophylls have been named *Antholittus Arberi* as a tribute to my former teacher.

The megasporophylls had a pinnate form also (Fig. 7 A); the side branches, which were not truly lateral,¹ but sprang from the upper side of the axis, terminated in ovaries with basal stigmas. Two genera can be recognized in Yorkshire named *Gristhorpia* and *Caytonia*, which differ in the shapes of the ovaries, in their stigmas, the thickness of the ovary wall, and the structure of the seeds. The wall of the ovary in both genera appears to have been a perfectly homogeneous structure, corresponding to a recurved and closed cupule rather than to a folded pinna with united margins. Its epidermal cells were quite uniform, and no trace of a suture can be found. On maceration the cuticle remains as a spherical envelope, and does not open out as in the case of a macerated follicle of *Caltha*. In *Caytonia* two rows of ovules were produced internally along a line running from the stalk of the ovary round its periphery, and the ovary might perhaps be interpreted as a recurved pinna with an expanded midrib, bearing ovules, and a much reduced lamina. It seems impossible to consider the seeds as marginal productions; they must have been borne on the midrib or median vascular bundle. Preference is given to the view that the ovary wall represents a closed cupule rather than a recurved pinna with an expanded midrib, because no traces of veins can be seen, and because this idea is supported by the new type of sporophyll, called *Unkomasia*, which will be mentioned later.

¹ This point was illustrated in my original paper (37), cf. Plate XII, Figs. 1, 4, and 5, but was not sufficiently appreciated when the description, p. 305, was written.

It could hardly be expected that a group of structures so novel and so unexpected would fail to raise doubts as to the validity of the original description, and as to the conclusions drawn. Thus Berry (5, p. 11) remarks, 'Should this author's conclusions be substantiated, and it seems wise to withhold judgement until they are fully demonstrated, they introduce us to the first really primitive angiosperms that have been discovered'. But the general facts of structure of the plant-remains included in this group are matters of observation rather than deduction; they can be verified by any one who is prepared to take the necessary trouble.¹ Certain important points, however, cannot be demonstrated by direct observation and involve the consideration of evidence. The most weighty points which have been raised are as follows:

(a) Was *Caytonia* really an angiosperm, or did the ovary become closed after fertilization of the ovules? (Cf. Kräusel (16, p. 98), In Engler's 'Pflanzenfamilien', 2nd edition, where the Caytoniales are placed among the gymnosperms.)

(b) Did the microsporophylls (*Antholithus Arberi*) really belong to the same type of plant as the megasporophylls (*Caytonia* and *Gristhorpia*)?

(c) Was *Sagenopteris* the leaf of this plant?

The view that these structures were gymnospermous is an assumption without foundation, and is opposed by direct evidence. The individual ovaries were closed from a very early stage, a point which can be readily determined, for had they been open, the fine mud of the matrix would have entered, and even the smallest ovaries contain no mineral matter. Some additional small specimens of young detached ovaries have recently been obtained by Dr. Harris from the Gristhorpe Bed by his method of bulk maceration. These are almost circular, 1.5 mm. in diameter, and show their papillate stigmas very clearly; they contain no matrix material.

In both *Caytonia* and *Gristhorpia* a distinct papillate stigma is found at the base of the ovary, where the tip of the recurved cupule joins the stalk. Pollen grains have been seen adhering to this stigma, though absent from the rest of the ovary wall. In two specimens which were carefully examined, the majority of the grains were of the winged type, though a few other grains, probably foreign pollen, were seen. Sections cut through the ovaries indicated that the cells of the stigmas became strongly cuticularized as growth proceeded, and it may be mentioned that the stigmas of several modern flowers become cuticularized in a similar way after pollination, though not so strongly, especially in the Cruciferae, Ranunculaceae, and Rosaceae.

The evidence for considering that the microsporophylls and the megasporophylls described belonged to plants of the same type is partly derived

¹ Most of the type specimens are now in the British Museum. Examples of fruits and seeds have been distributed to a number of other museums abroad.

from these pollen grains, but also from the structural and morphological features of each. It may be summarized as follows:

1. *Association.* *Antholithus Arberi* occurs in the same bed of shale in Cayton Bay in close proximity to *Gristhorpia* and *Caytonia*. Anthers and seeds referable to similar forms were found together in Scoresby Sound, East Greenland, by Dr. Harris (12, p. 77).

2. *Morphology.* Male and female sporophylls are both pinnate in general form, and show dichotomy at their apices. Lateral branches terminate in groups either of anthers, or of seeds enclosed in an ovary (cupule).

3. *Structure.* Epidermal cells (cuticles) of both sides of microsporophylls almost identical with the corresponding cells in *Gristhorpia*.

4. *Pollination.* Winged pollen grains characteristic of *A. Arberi* occur on the stigmatic surface of *Gristhorpia*.

There is also a strong presumption that these fertile structures belonged to the plants which bore the leaves long known as *Sagenopteris*, again for several reasons:

1. *Association:*

(a) Fruits referable to the Caytoniales have been found in Yorkshire, Greenland, and Sardinia (8, p. 386); in each place they are associated with *Sagenopteris* sp.

(b) Statistics of association in Yorkshire indicate a distinct correlation between leaves and reproductive structures.¹

(c) No reproductive structures have ever been found in spots in which *Sagenopteris* was not abundant.

2. *Morphology.* Sporophylls pinnate. Leaves usually have two pairs of pinnae close together.

3. *Structure.* Epidermal cells (cuticles) of sporophylls closely comparable with those of petioles of *Sagenopteris*.

In view of the opinions expressed by Zimmermann (46, p. 275) it is necessary to point out that the reference of *Sagenopteris* to the Hydropteridae and its comparison with *Marsilia* rest solely on a similarity in external form, a notoriously untrustworthy type of evidence. Reasons against such a relationship are: (a) Cuticles of *Marsilia*, like those of all ferns yet investigated, differ in chemical composition from those of *Sagenopteris* and do not resist maceration by strong acidic oxidizing agents. (b) The distribution of stomata is different in the two forms. There is no proof that the structures described as *Marsilia*-like sporocarps (11, p. 11, and 4, p. 329) were actually sporocarps, for no spores have been extracted from them.

¹ For table of numbers see Thomas (37), p. 332.

Comparison of the Caytoniales with the Flowering Plants.

The Caytoniales were certainly angiospermous, but the question now arises of their relationship, if any, to the flowering plants. At first sight it seems remote, but for that reason the question is more interesting.

The leaves in their general external form and in their cuticle characters may be compared with those of modern angiosperms, but they differ in their venation. In this respect they may be compared with certain immature leaves of modern plants where the small veins inside the primary network have not yet developed. The mature carpels of some species of *Aconitum* and *Delphinium* have a similar venation. Leaves with a comparable venation, but different in shape, have been found in Lower Cretaceous rocks in several places and identified as angiosperms (3, p. 494, and 18, p. 122).

Before attempting the comparison of the reproductive organs of the Caytoniales and flowering plants we must set aside all preconceptions as to the nature and origin of stamens and carpels based on the ideas of morphological categories which were current at the end of the last century. The elaborate and sometimes preposterous theories which were built up in the attempt to derive anthers, carpels, and ovules from expanded foliar structures, with fertile edges, can now be discarded and replaced by concepts based on the structures as we find them to-day and on the evidence of actual fossil plants.

It is very doubtful whether the view that the stamens and carpels are fertile leaves rests on any inductive basis. The fact that transitional forms are found between stamens and petals, or between carpels and leaf-like structures has always been taken as a proof that stamens and carpels have originated from leaves and might therefore revert to their original form. But there is no proof that this is a reversion. On the other hand, there is evidence in almost all the higher plants of the sterilization of structures originally fertile, and this affords a sounder basis for the interpretation of such structures as petaloid stamens.

We must now recognize that in the earliest land plants the terminal branches of the thallus were specialized for reproduction before any differentiation of the vegetative body into stem, root, or leaf had occurred. This view is not only supported by the recent extensive discoveries of early Devonian plants, and by researches on the Pteridophyta, such as Professor Bower's work on the Ferns, but it is in far greater accord with our knowledge of fossil gymnosperms than the older hypotheses.

The attempt of Dr. Zimmermann (46) to apply to the seed plants similar concepts based on the Psilophytales, probably marks the commencement of a new era in the morphology of the phanerogams. We now have a theory which gives a reasonable explanation of many of the known facts,

though doubtless it will require much modification as our knowledge increases.

When we consider the sporophylls of the gymnosperms from this standpoint we must recognize the force of Zimmermann's argument (46, p. 24) that the megasporophyll of *Ginkgo* represents the primitive type. Sporophylls, like foliage leaves, must be derived from specialized branch systems and the fertile terminal twigs tend to retain their radial symmetry while the vegetative parts may become dorsiventral. In some cases, as in the well-known pteridosperm *Lyginopteris* (*Lagenostoma*), the sporophyll is almost identical with an ordinary foliage leaf, but this cannot be regarded as an essential characteristic and there is no need to regard the sporophylls of the Caytoniales as reduced from some broad leafy form. The groups of synangia in *Crossotheca* and *Telangium* probably represent tufts of fertile branches, but we now know from the brilliant researches of Professor Halle on *Holcospermum* (*Rhabdocarpus*) *elongatum* Kidst., and on *Whittleseya*, that other pteridosperms possessed a different type of microsynangium.¹ In this there is a single fertile twig which is much enlarged and contains numerous longitudinal sporangia. The discovery of these Palaeozoic forms of microsynangia indicates the futility of trying to derive the angiosperm anther from a leaf with a flattened blade and marginal sporangia (45, p. 162).

There can be no doubt that a single synangium of *Antholithus Arberi* is closely comparable with the anther of a typical flowering plant, in external forms, in its mode of dehiscence, and in its possession of four longitudinal pollen sacs. On the other hand, there is much similarity between *A. Arberi* and some species of *Crossotheca* and *Telangium* in the general construction of the sporophyll.

The derivation of the stamen of a flowering plant from a structure of the type seen in these fossils requires the suppression of the vegetative part of the sporophyll and the reduction of the number of the synangia to one. This may appear to be an enormous change, but a precisely comparable one must have taken place in the case of *Wielandiella* among the Bennettitales. In the flower of this form Nathorst (21, p. 22) found a collar or whorl of sessile synangia round the lower part of the floral axis in place of the large sporophylls of the more typical members of the Bennettitales. In *Williamsonia spectabilis* (34, p. 230) and *Cycadeoidea* (44, p. 106) each sporophyll is pinnate and each pinna bears a large number of synangia, so that it is reasonable to suppose that in *Wielandiella* both the vegetative tissue and the number of synangia were greatly reduced.

It may well be that the aggregation of the sporophylls in the flower has been the main cause of their reduction in size and fertility, but even though this suggestion is regarded as a mere assumption it is far less

¹ Halle in Abstracts of Communications to the Fifth Botanical Congress, 1930, (47, p. 472).

speculative than many assumptions which have hitherto been held in connexion with the evolution of the stamen. We have, however, several cases among the flowering plants in which the stamens are still branched structures bearing numerous anthers. Thus in *Ricinus communis* the filament shows repeated dichotomy into more or less equal branches; the vascular strands fork at some distance below the point where the branches separate; each branch bears an anther at its apex. Such a stamen may well represent a primitive type.

The frequent occurrence of abnormal flowers in which the stamens bear ovules or the carpels give rise to anthers and pollen sacs (45, p. 182) would suggest that the microsporophylls and megasporophylls were originally homologous and similar structures. Probably the anthers of the male sporophylls correspond to the ovules of the female sporophylls. In the Caytoniales there is considerable similarity between the two kinds of sporophyll in general construction and in epidermal structure as well as in the position of the fertile segments.

Carpel morphology.

The megasporophylls of *Caytonia* and *Gristhorpia* are very comparable with those of some pteridosperms in their general form, but their morphological comparison with the carpels of the flowering plants seems at the outset very difficult. In order to attempt a comparison we need to know more about carpel morphology than can now be learnt from botanical literature. The most widely held view is that the angiosperm carpel represents a *simple* folded leaflike structure, bearing ovules on the infolded margins. This view originated in pre-evolution days when the question at issue was mainly that of the morphological category of the organ. The evidence adduced proved nothing more than the foliar character of the structure, which was held to be comparable with the megasporophyll of *Cycas*. But there is no proof that it represents a simple leaf with marginal ovules, and the comparison with *Cycas* breaks down as soon as the vascular structures are examined. The statements in some of the books are surprising and indicate the need for accurate information on this point. Velenovsky, for example, figures the hypothetical fertile leaf (Fig. 1) and states that the carpels of *Caltha* and *Helleborus* are constructed in this primitive form (39, p. 961). But when we examine the follicles of these plants we find a totally different structure. There are three main bundles running from base to apex, and Smith has shown that these arise from different points on the vascular system of the axis (31, p. 5), the same point has been shown very clearly in *Aquilegia* and other carpels by Eames (7, pp. 149-61). The two adaxial or ventral bundles run along the placentae, and the abaxial or dorsal bundle corresponds to the midrib of Velenovsky's figure. When we slit the carpels along the suture between the placental

bundles and bend back the two halves we can readily compare the vascular system of the supposed carpellary leaf with that of the hypothetical primitive type (Fig. 2). Carpels of *Caltha*, *Helleborus*, *Aquilegia*, *Aconitum*, and *Del-*

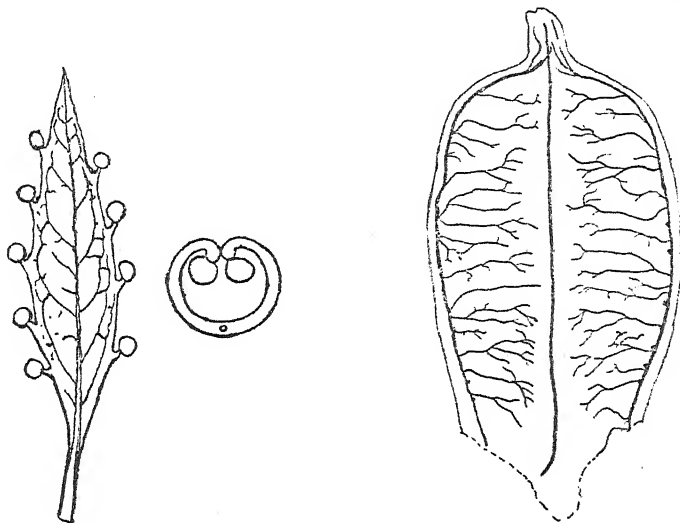


FIG. 1.

FIG. 2.

FIG. 1. Diagrams representing the hypothetical form of the primitive carpel according to the usual current view. Reproduced from Velenovsky (39), p. 960, Fig. 536. Compare with figs. 2 and 3 A.

FIG. 2. Follicle of *Caltha palustris* L. opened along the suture and flattened out to show the venation. The ovules are not drawn, but the points of their attachment to the placental bundles are indicated in some cases. From a photograph. $\times 4$.

pleinium were treated in this way, they were then cleared and stained, the ovules were removed, and they were mounted and photographed. Figures 2, 3 A-D, 4, 5, have been prepared by tracing over the veins and outline on photographic prints with waterproof ink and then bleaching out the silver image. It is seen at once that in the carpels of *Caltha* and *Helleborus* the dorsal bundle, which is supposed to correspond with the midrib, is simple and unbranched while all the lateral veins arise from the placental bundles. The stigma is supplied with veins from all the three main strands and not merely with the dorsal vein and its branches. In *Caltha* the lateral veins fork without anastomosing, in *Helleborus* we have a few fusions to give simple meshes, while in *Aquilegia* the lateral veins form a dense series curving upwards towards the dorsal bundle without uniting with it. In *Aconitum* and *Delphinium* we have many anastomoses and the dorsal vein gives off a few lateral branches which may unite with branches from the placental veins. On grounds of floral structure *Caltha* and *Helleborus* would be regarded as the more primitive and the open character of the lateral venation also indicates that *Caltha* is a primitive type. Here we have a structure which is in no way comparable with the megasporophyll of *Cycas*

and which seems to require some other morphological explanation. It would be interesting to know whether all 'valve' carpels can be derived from the *Caltha* type,¹ but the author has not been able to follow up this

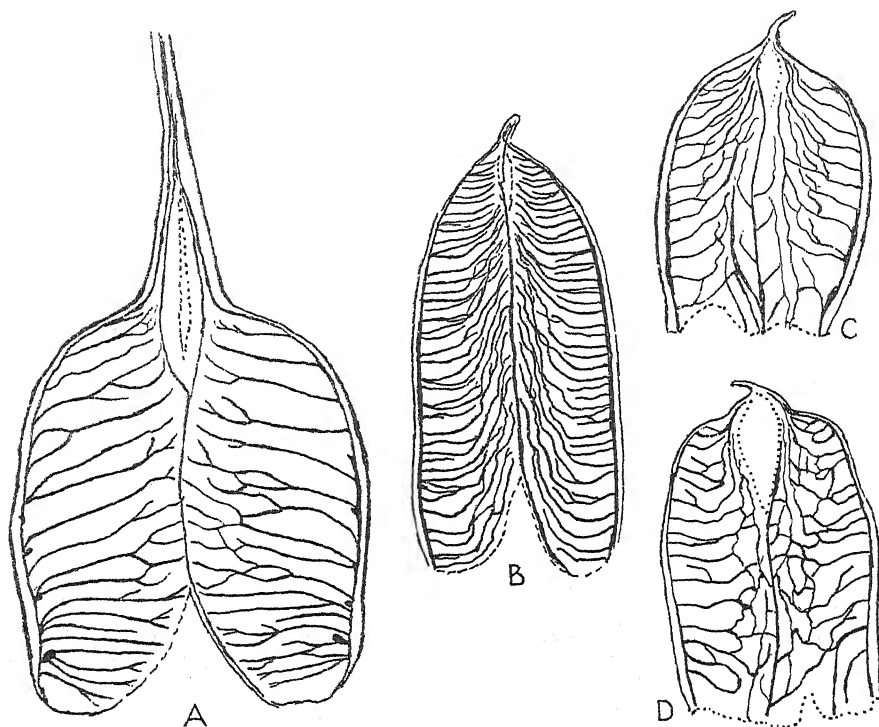


FIG. 3. A-D. Follicles of four genera of the Ranunculaceae opened out to show their venation. From photographs. A. *Helleborus orientalis* Lam. $\times 8/3$. B. *Aquilegia vulgaris* L. $\times 3$. C. *Delphinium*, garden hybrid. $\times 3$. D. *Aconitum Napellus* L. $\times 3$. Note that most of the secondary veins arise from the placental bundles. In the actinomorphic flowers, A and B, the dorsal bundles are simple. In the carpels C and D from zygomorphic flowers the venation is developing into a closed reticulum.

line of inquiry; two other examples may, however, be mentioned which lend some support to this idea. The sterile and open carpel of the cultivated double cherry, *Prunus avium*, which is shown in Fig. 4, also has a dorsal strand which is undivided and unbranched throughout most of its course while a profuse system of veins arises from a pair of veins which are here lateral rather than ventral.

The venation of the legume of *Galega officinalis* (Fig. 5) shows some interesting features. The dorsal vein is here the strongest and gives off numerous lateral branches, but these do not reach the margin and the two placental bundles produce their lateral branches as before. The lateral

¹ It is clear from the work of Miss Saunders that carpels in several other families, e.g. Sterculiaceae, have an essentially similar venation, though Miss Saunders places a different interpretation upon the results of her observations. Cf. Saunders (28), p. 106, Fig. 44.

branches from the dorsal and placental bundles anastomose, but the tripartite nature of the structure is clearly retained. This structure again affords very little support to the old hypothesis, and it can be regarded as derived from the type seen in *Delphinium*.

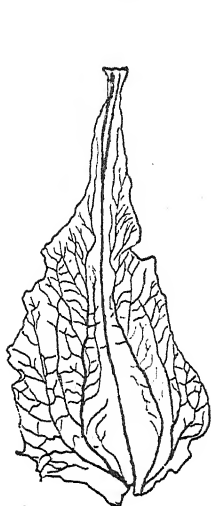


FIG. 4.



FIG. 5.

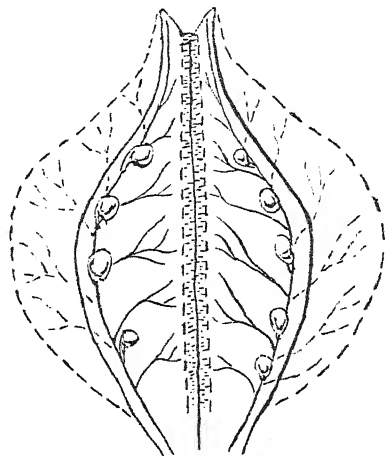


FIG. 6.

FIG. 4. Sterile open carpel of double flower of *Prunus avium* L. The dorsal bundle is almost unbranched, and the two sterile placental bundles give off a profuse system of secondary veins towards the free margins. From a photograph. $\times 7$.

FIG. 5. Legume of *Galega officinalis* L. showing numerous secondary veins given off from the dorsal bundle in addition to those arising from the placental bundles. The three series of veins are quite distinct although connected. From a photograph. $\times 5$.

FIG. 6. Diagram to illustrate the hypothesis of the derivation of a *Caltha* follicle from a palmate sporophyll. The portion thought to be derived from a dorsal segment is shaded. The parts of the hypothetical lateral segments aborted owing to contact are indicated by broken lines.

It has been suggested that the character of the secondary venation has no morphological significance but depends on chance or on the relative size and importance of the dorsal and ventral veins in the nutrition of the carpels and ovules. But the facts of heredity, and the examples figured, indicate that the general pattern of the system of veins depends on something more fundamental. Variations possibly due to differences in nutrition certainly exist, as have been observed in the annual and perennial species of *Delphinium*, but they do not cause a transformation in the pattern of the venation.

In seeking some explanation of the structures described above it would seem that there is in the venation a clear suggestion of the derivation of the apparently simple follicle from a compound structure. The three main veins may well represent the midribs of three segments of a palmate sporophyll (see Fig. 6), the dorsal bundle representing a central sterile segment while the placental bundles represent the *midribs* of the lateral fertile

segments which bore ovules along their centres and also possessed laminae with lateral veins. The structure of *Caltha* would suggest that the sterile central segment was originally narrow and simple. If these three segments became fused and inrolled to form a closed structure the laminae on the adjacent sides of the fertile segments might fail to develop, so allowing the placental midribs to lie side by side and giving the appearance of unilateral development. Such asymmetrical or unilateral development can easily be produced by light contact during growth; it may be seen in almost every year in the growth of some of the fruits on a plum tree which has not been thinned. Fruits which have been produced from adjacent flowers and which come into contact generally fail to expand and become fused along the contiguous side while developing normally on their other side. Should this suggestion be correct we should expect to find that the lateral segments would produce a symmetrical lamina on either side of the placental bundle if for some reason the carpel were prevented from closing. This is exactly what we find in the open sterile carpels of the double cherry (see Fig. 4). These structures must be regarded as true open carpels; they not only arise at the apex of the flower in the place of the carpels but they also have stigmas at their apices.

Since this view of original compound nature of the follicle was formulated by the author it has appeared that at least two other botanists have arrived at a somewhat similar conclusion. Professor Vuillemin in his book on vegetable teratology (40, p. 58) put forward the view that the carpel is composed of two heterogeneous members, a phyllome and a frondome, associated in development. He based his theory on certain points in floral morphology and on a number of abnormalities, including a case described by Baillon (1880) where a virescent flower of *Delphinium consolida* had its placentas free and inside the open carpel. In the discussion on the subject of the present paper at the Fifth International Botanical Congress at Cambridge, Professor Eames stated¹ that his work on the comparative structure of carpels had led him to the view that the primitive carpel of the flowering plants was a palmate structure. The whole question is one which should engage the attention of botanists who are concerned with the study of recent angiospermous flowers.

Now, if the original megasporophyll of the flowering plants was a compound structure of the type suggested, it may be reasonably derived from an organ of the type seen in *Caytonia* and *Gristhorpia*. The series of drawings shown in Fig. 7 is an attempt to indicate a possible method of deriving the *Caltha* carpel from the megasporophyll of the Caytoniales.

This series commences with a young sporophyll of the *Gristhorpia* type (A), as it is more satisfactory to base ideas on a known structure than

¹ See Discussion on the Antiquity of Angiosperms. Report of the Fifth International Botanical Congress (47, p. 466); also the fuller statement (7, p. 153).

on a purely hypothetical one. In Fig. 7 A' a pair of the primary ovaries is seen from one side. The first suggested change is the reduction of the

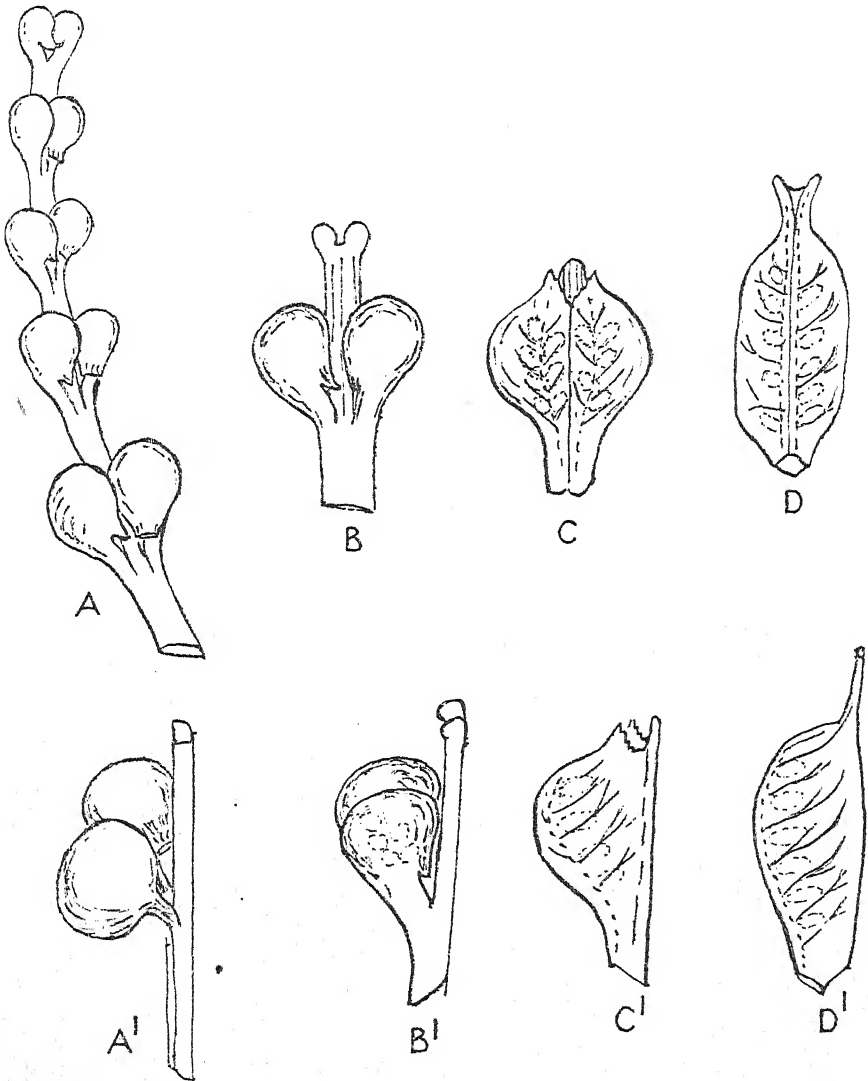


FIG. 7. Diagrams illustrating the suggested mode of derivation of the follicle (D, D') from a megasporophyll of the *Gristhorpia* type (A, A'). Above, the actual and hypothetical intermediate structures (B, C) seen from the front. Below, the corresponding structures seen from the side. (See text.)

ovaries to a single pair (B, B'), this reduction is similar to that which has been suggested above for the microsporophylls on the analogy of the Bennettitales, and is also supported by the consideration of some of the specimens of *Umkomasia* mentioned below. Small aborted ovaries may

have remained on the axis, and in some cases may have influenced the subsequent formation of the stigma.¹ The two fertile ovaries may now have become fused by the shortening of their pedicels and the reduction of the vegetative tissue.² The form of the youngest ovaries of *Gristhorpia* suggests that the ovary wall arose on one side of the stalk or pedicel, and as it grew upwards it curved over—owing perhaps to an inherited tendency to circinate growth—and formed a hoodlike structure; its apex eventually reached the stalk again and became differentiated into the stigma. If the pedicels were reduced the walls of an opposite pair of ovaries might have come into contact during development, and might have been mutually prevented from assuming their recurved form. Thus instead of two ovaries with basal stigmas we should have a single ovary with an apical stigma and two rows of ovules (Fig. C). The reduced sporophyll axis may also have become involved in this fusion (C'), while the two rows of ovules would tend to approach the ventral suture (D), for, as seen in many modern instances, the ovary walls would fail to expand along the line of contact.

A stigma which has originated in this way would probably show a decided tendency to fork into two equal parts; this is a feature which is very commonly found, but which is an anomaly in an angiosperm organ derived from the apex of a simple foliar structure in the way formerly postulated.

Thus, starting from a known type of fossil sporophyll and postulating little but the operation of the processes of reduction and fusion, we reach a follicle of the *Caltha* type. Can any other suggestion for the evolution of this structure be made without assumptions that are more speculative?

It must be clearly pointed out that the author does not consider the Ranunculaceae as descended from the Caytoniales. In the scheme outlined above, the *Gristhorpia* sporophyll is used only as an example of a compound structure bearing ovules enclosed in ovaries whose walls seem comparable to cupules which have become recurved on one side and formed stigmas. There is some reason to suppose that structures of similar construction may have occurred in other groups, at present unknown, and from one or more of these the flowering plants may be descended.

The Caytoniales and the Pteridosperms.

The general external morphology of the Caytoniales and the structure of their seeds, in so far as it is preserved, suggest the derivation of this group from the Palaeozoic pteridosperms. But this suggestion raises two ques-

¹ Cf. stigmas figured by Goebel (10), p. 1638, Fig. 1515 and Fig. 1514.

² Professor Eames has suggested to me that this may perhaps be compared with the formation of the sporocarp in *Marsilia*.

tions. (1) Is there any evidence of the existence of pteridospermous plants with seeds enclosed in a cupule which might have become closed to form an ovary of the *Gristhorpia* type? (2) Is it not probable that the Caytoniales were an isolated group in which we see a particular case of the evolution of angiospermy that has no significance in connexion with the evolution of the flowering plants? To answer these questions we need evidence, (a) of the former existence of pteridosperms with an open cupule comparable to the ovary wall of the Caytoniales, and (b) of the occurrence of similar megasporophylls with seeds enclosed in cupules in other groups of fossil plants not closely related to the Caytoniales.

When we remember the number of different types of petrified stems and of fronds which must be referred to the Pteridospermophyta it is clear that our present knowledge of the reproductive structures in this group is very meagre. We do know, however, of the case of *Gnetopsis* in which two to four seeds with very long micropyles were produced within a large investing cupule (23, p. 180, and 22, p. 31), but there is no evidence of it becoming recurved or closed.

In 1929 a new form of pteridosperm of exceptional interest was discovered by the author, and, although its investigation is not yet complete, sufficient progress has been made to provide material for a preliminary account of its main features. In the Triassic rocks of the Southern Hemisphere well preserved plant-remains have been discovered, especially in the Molteno Beds of Natal, South Africa, and in the Narrabeen Beds and Ipswich Series of Eastern Australia. In these deposits are found many thick, fern-like fronds which have usually been called *Thinnfeldia*, but for which Gothan's name of *Dicroidium* is to be preferred. Associated with these are the remains of seed-bearing megasporophylls of the form shown in Fig. 8. These structures were branched, and the sub-opposite terminal branches bore recurved cupules enclosing one (or possibly more) seeds. The cupules were open, and in some cases the micropyles of the enclosed seeds are seen to project from them (Fig. 8, B). In older examples a large part of the seed with its characteristic curved and bifid micropyle projects (Fig. 8, C). It seems probable that the seeds fell out of the cupules when mature, as isolated seeds and empty cupules are found. In some specimens the terminal cupules appear more or less fused together, while in others some of the branches appear aborted. This type has been named *Umkomasia*, from its occurrence in the upper Umkomaas valley in Natal, where well preserved specimens were found in the Molteno Series of beds. Fragmentary specimens had previously been described from Australia by Walkom (41, p. 26, Pl. 8, figs 7 & 9, and 42, p. 222, Pl. XXXI, figs. 7-9) as plants of doubtful affinities. Evidence has been obtained from (a) association, (b) the external appearance of the stalks as viewed under the binocular microscope, (c) the structure of the cuticle and the stomata, which

seems to show that *Umkomasia* belonged to the plants whose leaves are known as *Dicroidium*. The cuticles and stomata of these leaves indicate that their affinities were with the gymnosperms rather than with the ferns,

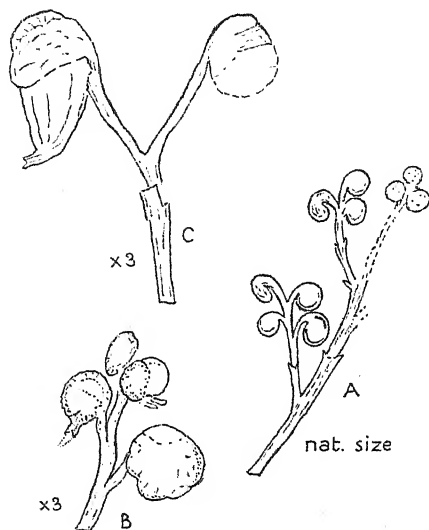


FIG. 8. *Umkomasia*, gen. nov. Drawings of specimens of different species and ages of growth. A. Part of a sporophyll showing form and arrangement of the cupules. No seeds are visible. B. Young pinna with a large cupule to the right below, and two cupules above with projecting micropyles of the enclosed seeds. $\times 3$. C. Older specimen with large attached seed and broken remains of cupules. $\times 3$.

and thus it is highly probable that we have in these fossils the remains of a new type of pteridosperm from the Trias. While this new type is far removed from the Caytoniales, the general appearance of the megasporophylls suggests that the *Gristhorpia* ovary might have been derived from a cupule of the *Umkomasia* type.

The discovery of *Caytonia* and *Umkomasia* are of some interest in connexion with the question of the mode of origin of angiospermy and the evolution of the stigma. The current idea of the carpel as an infolded leaf furnishes no clue to the appearance of the stigma with its characteristic receptive surface, but it is clear that the closure of the carpel must have been preceded by or accompanied by the evolution of the stigma. Now in some of my specimens of *Umkomasia* we see a cupule, like the ovary wall of *Gristhorpia*, but with the micropyle of the seed projecting through an opening in the position of the *Gristhorpia* stigma. It seems reasonable to suggest that the first stage towards angiospermy was the closing of the cupular envelope around or upon the micropyles. We know that in *Gnetum* a considerable amount of mucilage uncutinized is exuded from the micropyles of the seeds when ripe for pollination (32, p. 119, and 33, p. 40), and the same thing may have occurred in our fossil forms. It is just

possible that the presence of such fluid may have affected the development of the cells of the cupule surrounding the micropyle, and have led to their acquiring a similar secretory power with a delay in cutinization. When germination of the pollen habitually started on this stigmatic surface, the complete closure of the ovary could follow.

This suggested mode of origin of the stigma is obviously mere speculation, but it may be worthy of mention because it would account for that curious feature in the flowering plants, the anatropous ovule. There seems to be at present no adequate explanation of why the majority of flowering plants should possess anatropous ovules while orthotropous ovules are rare. It may well be a case of a character which was evolved early in the history of the angiosperm line, and which has been usually retained in spite of great changes in the form of the ovary. On the view put forward above, the micropyle would have originally pointed downwards along a line more or less parallel to the placenta or stalk of the ovary, owing to the overarching of the cupule. This position might be attained by the downward growth of the ovule as a whole, or by the curvature of a long micropylar tube, or by the ovule assuming the anatropous position. Since the whole 'ovary' had the shape of an anatropous ovule the chances that the enclosed ovules would develop a corresponding shape are considerable. After the stigma had been evolved there would be no reason why the form of the ovule should change, especially if chalazogamy became the rule.

Origin of the Flowering Plants.

Some reasons have been given above for considering that the Caytoniales may have been derived from the pteridosperms. On the evidence of anatomical structure of the vegetative organs, together with that derived from the comparison of seed structure, it would seem reasonable to trace the Bennettitales back to the same group. We see in the Caytoniales the development of angiospermous sporophylls and in the Bennettitales the production of condensed groups of fertile structures or flowers. What is more probable than the former existence of some other group or groups, at present unknown, in which sporophylls somewhat of the *Caytonia* type were grouped close together on branch endings in the same manner as is seen in the Bennettitales? Several types of our modern flowers might have evolved from such forms by reduction in the number of parts and by fusions of the type which has already been indicated.

An attempt is made in Fig. 9 to express in a diagrammatic form this view of the relationships of the flowering plants to the various groups mentioned above and to some other gymnosperms. It is only a rough sketch, but it shows the ages of the various groups so far as these are known. The intervals between the horizontal lines represent time intervals

of approximately thirty millions of years, according to the conclusions of Professor Holmes (13, p. 78). The full radiating lines indicate that we

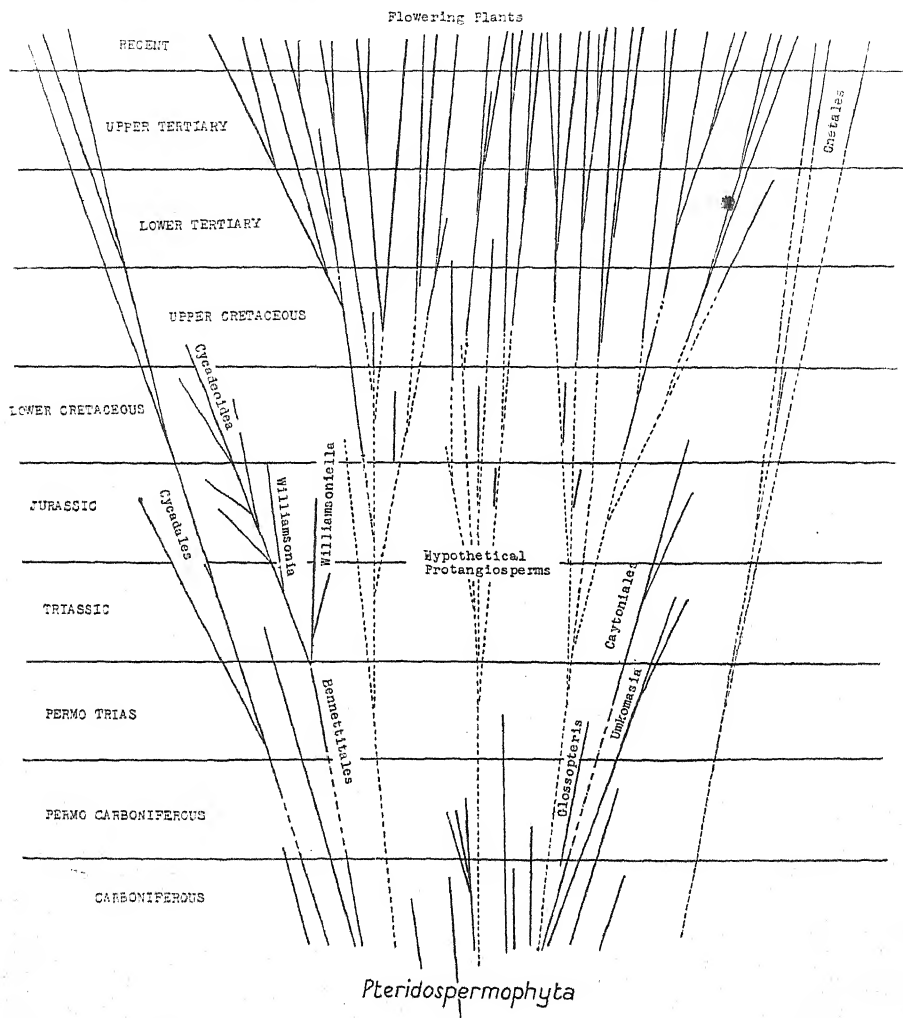


FIG. 9. Diagram showing the probable distribution of the flowering plants and allied groups of gymnosperms in time. The intervals between the horizontal lines possibly represent about thirty million years. The full lines represent the probable duration of the groups; the broken lines represent hypothetical interconnexions.

have some evidence of the existence of the group named during the period occupied by the line, while the dotted lines show hypothetical connexions between the various groups. The number of lines drawn for the flowering plants, protangiosperms and pteridosperms, was chosen for convenience in constructing the diagram, and a much greater number of series probably existed.

In making this suggestion of the ultimate derivation of the flowering plants from the pteridosperms, it is scarcely necessary to recall the fact that the latter group included an exceptionally large number of diverse forms in Upper Palaeozoic times, and may well have given rise to many series of descendants. Dr. D. H. Scott has demonstrated how varied was the structure of the early pteridosperm stems, and though at first sight these do not suggest any angiosperm affinities, yet several of them would pass for angiosperms if deprived of their centripetal wood and provided with vessels. There is good evidence that centripetal wood has in all other groups tended to disappear in the course of evolution, and there is at least some evidence of the comparatively late appearance of vessels in xylem, originally composed mainly of tracheides. Thus our general hypothesis is not unreasonable when applied to vegetative structures.

We must remember, however, that most of our present knowledge of the pteridosperms is derived from the fossil floras in the Upper Palaeozoic rocks of Europe. It is highly probable that most, if not all, of the mesophytic plants in this region were exterminated during the long and severe desiccation which seems to have occurred in late Permian and early Triassic times. Thus, the suggested angiosperm ancestors will probably not be found among our British pteridosperms.

If we knew more of the plants of the regions which remained moist and cool during this adverse period, we might find forms which were nearer to the main line of descent of the flowering plants. It is for this reason that fossils like *Glossopteris*, *Gangamopteris*, *Vertebraria*, and *Belemnopteris* are of special interest, for they occur in regions which probably escaped severe desiccation before the later part of the Triassic period when parts of the Northern Hemisphere were becoming once more clothed with a luxuriant vegetation. I have been tempted to suggest a phyletic connexion between *Glossopteris* and the Caytoniales (37, p. 354). *Glossopteris* is probably a pteridosperm, but our knowledge of its structure and reproduction is still so incomplete that the suggestion is little more than a speculation, and we must await further discoveries. Certain information that has recently been obtained is not unfavourable to the suggestion. Professor Sahni (25, p. 277, Pl. 17, figs. 1-3) has described the cuticle of a species of *Glossopteris* from India which is not unlike that of *Sagenopteris*. The wood of *Vertebraria* has been found to possess tracheides of a gymnospermous type. The leaves of *Glossopteris* were borne in whorls on the stems in some species. Winged microspores similar to those of *Antholithus Arberi* have been isolated by my wife from the shale of a Lower Beaufort plant-bed found at Lidgetton, Natal, which contained only the remains of *Glossopteris* leaves and *Phyllothea* leaves and stems.

CONCLUSION.

In this paper the author has dealt with the subject from the standpoint of the palaeobotanist working with plants and structures which actually existed in the past. Believing that it is useless to expect to find among Upper Palaeozoic and Lower Mesozoic floras plants with the characteristic features of modern flowering plants, the attempt has been made to interpret some features of recent flowers in terms of the fossil plants known to us. It is thought that if many of the traditional (and probably quite unsound) ideas of morphology are discarded, and the flowering plants are reinvestigated in the light of the ancient gymnosperms, much progress may be made.

The views put forward above, especially in regard to the origin of the carpel, may be found to require considerable modification in the future, but they constitute a challenge to the floral morphologist. There are many points in the structure of the gynaecium of modern flowers which at present seem inexplicable, but which may receive illumination by the application of some hypothesis such as that now suggested. For example, when we look at the development and form of the carpels in the Rosaceae (15), we find a number of cases (*Alchemilla*, *Comarum*, *Coleogyne*, *Kerria*, and *Nevinsia*) in which the style springs from the side of the ovary and not from its summit. In the case of *Alchemilla arvensis*, where the development of the carpel had been investigated by Murbeck (19, p. 3, figs. 1-5), the carpel wall with the style and stigma curves over the primordium of the ovule in the same way as the ovary wall must have developed in *Gristhorpia*. Then there is the question of the form and structure of stigmas, which seems to have received so little attention. Several of the forms described by Goebel (10, p. 1635) might be readily explained on the lines of my hypothesis, but are very difficult to understand otherwise.

The question of carpel polymorphism may also receive a contribution from the foregoing discussion. If the carpel has arisen as has been suggested, we might expect to find several forms of carpels. In addition to the ordinary 'valve' type, it may have happened that in some plants carpels developed from structures like a single *Gristhorpia* ovary, when their ovules would be borne along the midrib, as in Miss Saunders's solid and semi-solid types (27, p. 125). Again, it is not impossible that in other cases a campanulate cupule may have become constricted at the apex around a single terminal (orthotropous) ovule.

It is probable that objections will be raised which have not been foreseen by the author, but if these lead to the establishment of proved facts to replace some of the unsupported statements or half-truths which are now current, a service will be done to botanical science.

There still remains the problem of the rise to dominance of the angiosperms. In Jurassic times the Caytoniales seem to have been the only

angiospermous types in the delta floras, though there may well have been flowering plants living on drier ground; during the Lower Cretaceous the possible angiospermous types still formed an insignificant element in the floras which have been preserved, but in the Upper Cretaceous period the flowering plants formed 80 to 90 per cent. of the species preserved in most of the floras known. Thus, even assuming that the angiosperms are much older than has been previously supposed, we still have to explain this sudden increase and rise to dominance. There seems to be no evidence of climatic changes which would favour the angiosperms at the expense of the other elements in the floras, such as those which brought about the extinction of so many types at the close of the Palaeozoic era. But the possibility of climatic change as a factor must not be neglected. At present it seems preferable to regard this floristic revolution as due to the acquisition by the angiosperms of some new characters which greatly increased their rate of reproduction, their vigour, and their tendency to vary. Now the closing of the ovary must have led to an increase in the rate of reproduction, ovules would be more quickly matured and would probably be more rapidly fertilized, while fleshy fruits as seen in the Caytoniales could be widely and effectively dispersed by birds. Birds were undoubtedly evolved during the close of the Mesozoic period, and flower-visiting insects probably appeared about the same time. Rapidity of reproduction would increase the chances of mutation, while the evolution of the apical stigma would make insect pollination more effective. It is just possible that the almost contemporaneous evolution of birds, flower-visiting insects, and typical angiosperm flowers would account for the apparently rapid change in the world's floras.

SUMMARY.

1. There seems to be little prospect of solving the problem of the origin of the angiosperms from the comparative study of modern forms, and we must search through the remains of fossil floras for clues to possible solutions.

2. While the flowering plants can be traced back to the Jurassic period, none of the early forms now known provide evidence of their ancestral types.

3. All the known megaphyllous gymnosperms of the Early Mesozoic must be scrutinized with a view to the detection of general evolutionary tendencies leading towards the flowering plants.

4. The Bennettitales show the restriction of the fertile members to shoots of limited growth and the formation of flowerlike structures, together with considerable reduction in the size and fertility of the individual lateral members (sporophylls).

5. The Caytoniales had evolved angiospermy in Lower Jurassic times,

apparently by the closing of a cupule-like envelope around the ovules and the formation of a stigma. Antherlike synangia were also present, though the sporophylls show no signs of aggregation or reduction. The Caytoniales thus show similarity to the pteridosperms and to the flowering plants.

6. The evidence on which the various remains have been associated together in the Caytoniales group is reviewed and certain criticisms are dealt with.

7. The leaves of the Caytoniales (*Sagenopteris*) may reasonably be considered as protangiospermous, and suggestive comparisons can be made of the reproductive structures with those typical of modern flowers if we abandon the old views of floral morphology. Antherlike structures can be found among very early gymnosperms, and there is no evidence of their ever being flattened foliar organs. The view that the simple angiospermous carpel was derived from a structure like the *Cycas* megasporophyll is regarded as entirely without foundation or justification.

8. The study of the venation of the follicles of *Caltha* and other members of the Ranunculaceae brings out some unexpected features hitherto undescribed. These suggest the derivation of the carpel from a compound sporophyll.

9. The suggested connexion of the Caytoniales with the pteridosperms is supported by the discovery of a new Lower Mesozoic pteridospermous type named *Umkomasia*, of which a preliminary description is given. This form also suggests that developments in the direction of angiospermy may have occurred in several distinct groups of pteridosperms.

10. The flowering plants are considered to be derived from one or more groups of pteridosperms intermediate between those which gave rise to the Bennettitales and Caytoniales. This view leads to suggestions as to the origin and morphology of the stigma, and as to the significance of the anatropous ovule.

11. The rapid differentiation and spread of the angiosperms is regarded as the result of the development of a mode of reproduction which was much quicker and more efficient than that possessed by other contemporary plants.

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Studies of the Physiological Importance of the Mineral Elements in Plants.

III. A Study by Microchemical Methods of the Distribution of Potassium in the Potato Plant.

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With seven Figures in the Text.

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INTRODUCTION.

IN spite of a considerable amount of work on the mineral requirements of plants, we still lack exact knowledge of the function of many of the essential elements. In the case of potassium our ignorance is particularly marked, possibly because it does not enter into organic combination in the plant.

Hitherto, the most usual method of approaching the problem of the determination of the part played by potassium in plant metabolism has been to examine the effect of a deficiency of potassium in the nutrient medium. Such studies are those of Reed (16), Briggs (1), Maskell (11), Gregory and Richards (3), and James (5).

Another method of approaching the problem of the importance of this element is to follow the distribution of potassium, and its association with organic materials, as well as its movement in the tissues and organs of the plant body. Such an approach to this problem was suggested to me by Dr. W. O. James, under whose direction the work to be described was carried out.

The present paper reports the first part of the investigation, consisting of a microchemical examination of all parts of the potato plant. Investigations into the presence of potassium in the cells and tissues of various plants and animals, as determined by microchemical methods, have previously been carried out by Macallum (9), Weevers (22), Lloyd (8), and Dowding (2); while other workers, notably de Vries (19), have determined by microchemical methods the distribution of organic substances such as starch, sugars, and proteins in the plant. No attempt seems to have been made, however, to combine a study of potassium distribution with that of organic materials in the tissues of a plant at progressive stages of its growth. This is here attempted in the case of the potato plant.

MATERIALS AND METHODS.

Plants used.

The potato was the plant selected for investigation, since its richness in potassium should allow that element to be traced without difficulty. Three sets of plants, all of the variety Majestic, were grown, both from tubers and seeds, as follows: firstly, a crop of about 150 plants was raised in an ordinary garden plot, and used to trace the normal distribution of potassium; secondly, a series of plants was raised in carefully washed, sterile, and potash free sand, in order to determine differences in distribution consequent upon potassium starvation; the third set of plants, grown in a dark chamber, was used in tracing any variation in the distribution of potassium associated with etiolation.

The microchemical tests for potassium and organic substances.

With one or two slight modifications, the method described by Macallum (9), and known as the cobalt hexanitrite reaction, was used to detect potassium.

The presence of protein was demonstrated by means of Millon's reagent; that of starch by iodine in potassium iodide; and of reducing sugar by Fehling's solutions. In some of the earlier work Mangham's (10) osazone method was also used in an attempt to determine the localization of sugars, but the results were unsatisfactory, and so this method was abandoned.

DISTRIBUTION OF POTASSIUM IN THE CELL.

Inside the cell the potassium precipitate seemed to be localized in the cytoplasm and vacuoles either as small round well-defined granules of various sizes, or as very much smaller particles appearing either scattered throughout the cytoplasm or collected together as large black masses, unevenly distributed, but very often attached to the surface membrane of bodies such as plastids and nuclei.

Never in any cell from any part of the plant was the nucleus found to contain the black precipitate of potassium, although in several cases it had stained a dirty brownish-black; this was probably a secondary effect caused by the reagent.

THE DISTRIBUTION OF POTASSIUM THROUGHOUT THE PLANT.

Tuber.

A potato tuber is a storage organ which represents morphologically the greatly swollen tip of an underground stem; its anatomical structure is normal except for an increase in the parenchymatous ground tissue of inner cortex and medulla.

Sections of unsprouted and sprouted tubers tested with the cobalt hexanitrite reagent showed that all cells except those of the cork layers contained potassium; it was abundant, for example, in all storage cells and in the small cells in the region of an eye, but less concentrated relatively in the vascular strands, of which the parenchyma and phloem elements contained the most precipitate.

Starch, the chief storage material of the tuber, was located almost entirely in the large inner cortical and medullary cells. It was absent from the periderm, vascular strands, and practically absent from the neighbourhood of the 'eyes' and the two to three outer layers of the cortex.

Protein, on the other hand, had a distribution complementary to that of starch, being most densely located where starch was scanty, namely, in

the region of the eye, vascular strands, and especially in the outermost layers of the cortex just beneath the cork.

At the time the tuber was examined (November) sugar was present in nearly every cell. A tuber at the beginning of its rest period is, however, practically without sugar.

Formation of young tubers.

In an endeavour to discover some of the changes which occur during the formation of a young tuber, a special examination was made of tuber-forming stems from the following three sets of plants: normal light-grown plants, etiolated plants grown from whole tubers, and etiolated plants grown from small pieces of tuber. The tubers examined were formed at the tips of the lateral shoots; the stage of development varied from the first indication of a swelling to a tuber four times the diameter of the stem; the diameter of the tubers varied from 5 to 15 mm.

The chief anatomical change was an increase in the number of parenchymatous cells of cortex and medulla, especially of the latter, and with this increase in the ground tissue the ring of vascular bundles tended to separate into small, rather isolated strands. A periderm replaced the epidermis, the phellogen having arisen in the epidermis itself.

As regards the actual distribution of potassium and organic substances: it was found that in the young tuber-forming stem, unlike a non-tuber-forming stem, starch was deposited in considerable quantities in the cortex and medulla right behind, but not actually at, the growing apex. As tuberization advanced the starch spread from the apex down a few layers of the inner cortex, and then accumulated in the base of the swelling region; finally, all the cells of the middle and inner cortex were filled with starch (Fig. 1, A-C).

Potassium and protein both appeared to be present in greater quantity than in the normal stem, and the increase, especially of potassium, in the starch storage cells was so considerable that gradually the dense localization in the outer cortex and vascular tissue became less distinct (Fig. 1, D-F).

Tubers formed on the laterals of etiolated plants grown from a small piece of tuber and in sand, in contrast to the other two sets, contained very little protein indeed, and starch was absent from the stem region below the tuber, although it was present in considerable quantity in the tuber itself. This agrees with the results to be given for such a plant in revealing a further instance of protein starvation.

The quantity of sugar seemed to vary from tuber to tuber, probably owing to a variation in the rate of supply to the tuber and of synthesis into starch of this material.

The young sprout.

The sprouts were obtained from sets of six Majestic tubers grown in pans of sawdust in a greenhouse. Planted 2 days after their 'eyes' were noticed to have begun swelling; they were removed for examination at the end of 14, 21, and 25 days.

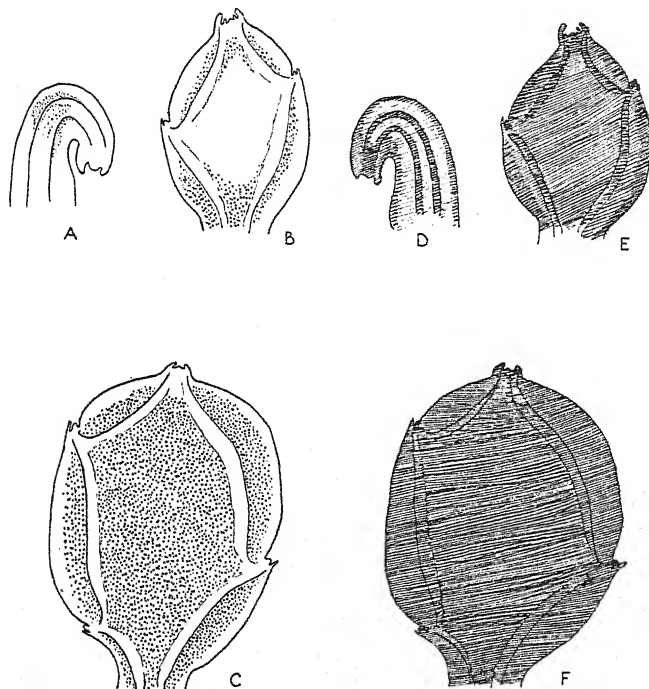


FIG. 1. A-F. Longitudinal section of a young tuber-forming stem showing distribution of starch and potassium. A-C show starch distribution. D-F show potassium distribution. A and D, stem with only a faint indication of swelling. B and E, tuber swollen to twice the diameter of the stem. C and F, tuber 3-4 times stem diameter.

The smallest sprout examined was 8 mm. long after 14 days; the potassium precipitate seemed to be as dense in it as in the tuber, and evenly distributed. After 21 days, when the sprout had increased in length to 14 mm., a localization of the potassium became apparent: although present in all cells it was concentrated, especially in the growing apex, in the vascular strands, in the outer regions of the cortex, in outgoing rootlets, and in epidermal hairs. The inner cortex and medulla appeared to contain much less, although there was an accumulation again in these regions at the base of the sprout as it merged into the tuber (Fig. 2, A).

The distribution of protein in the young sprout was found to be almost identical with that of potassium (Fig. 2, A).

All cells, except those in the apical 5 mm. of the sprout, contained

sugar (Fig. 2, C); it was probably as a result of this that starch was deposited in considerable quantity in the parenchyma cells at this early stage (Fig. 2, B), but the starch was only transitory, disappearing as the sprout elongated.

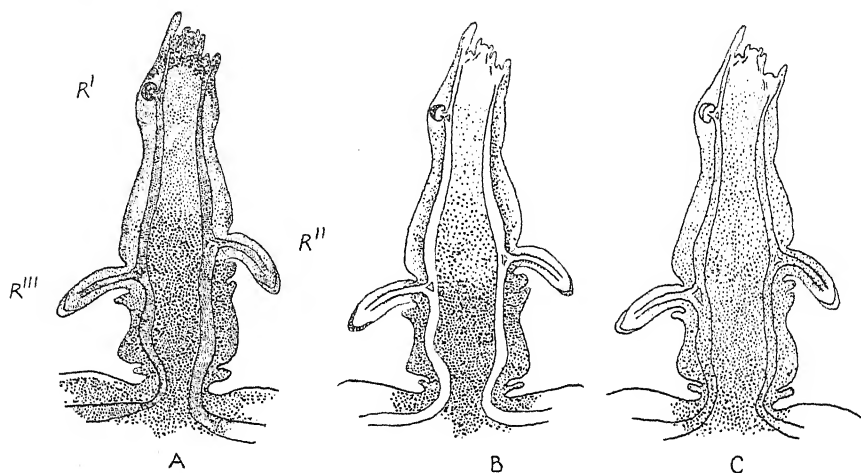


FIG. 2. A-C. Longitudinal section of a young sprout 14 mm. long showing distribution of (A) potassium and protein, (B) starch, (C) sugar. R' , R'' , R''' , adventitious roots. The tissue at the base of the sprout is that of the tuber.

The roots of the sprout are of the adventitious type. A longitudinal section, through the young root still inside the cortex of the sprout, showed that potassium and protein were present in large amounts in all cells (Fig. 3, A and B). The root-cap was densely filled with starch in contrast to the lack of this reserve in the surrounding cortical cells. Starch grains were also present in several layers of cells in the region of the endodermis and in the central strands of cells in the cylinder (Fig. 3, B).

In a young root 5 mm. long, which had emerged from the sprout, the potassium was most concentrated in the growing tip, with the exception of the root-cap cells, which were poor in potassium (Fig. 3, C), and in the vascular cylinder. Measurements were taken of the extent of the apical zone of potassium in this and other roots, and are given in Table I.

In an older root, 35 mm. long, there appeared to be considerably less potassium in all the cells behind the apical 5 mm., except in the phloem strands (Fig. 3, E). The precipitate was slightly greater in the outer cortical region as compared with the inner. This apparent decrease in the amount of potassium per cell can probably be accounted for by the fact that the increase in size of the cells due to vacuolation was not accompanied by a proportional increase in potassium per cell.

In the last two roots described, the protein localization, as in the stem, was found to coincide almost exactly with that of potassium (Fig. 3,

D, G). Measurements of the length of the apical region of the root-tip, showing richness in protein, were made and compared with those already obtained for potassium (Table I). The figures show that the protein zone is always a little less in length than the potassium zone.

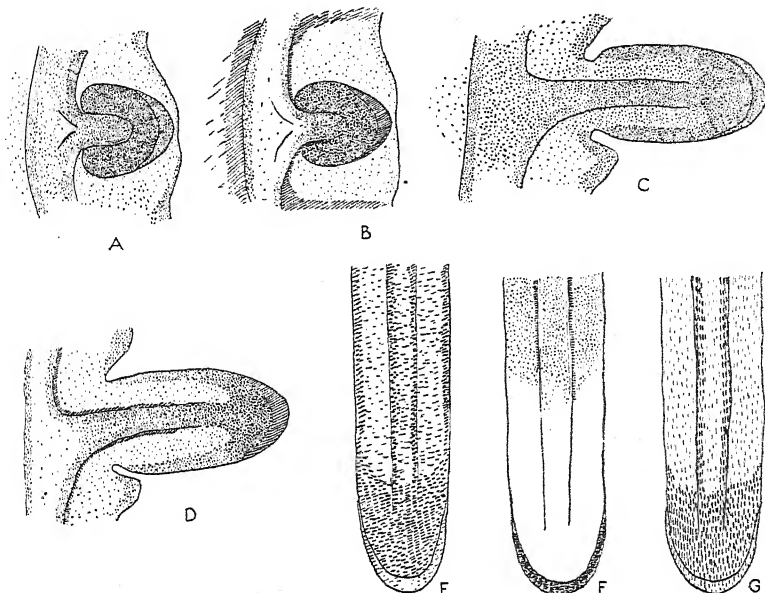


FIG. 3. A-G. Distribution of potassium, protein, and starch in the adventitious roots of the sprout. A and B. Longitudinal section through a root still inside the cortex of the sprout. A, potassium, B, protein—vertical shading, starch—horizontal shading. C and D. Longitudinal section of young root 5 mm. long which has emerged from the sprout. C, potassium, D, protein and starch represented as in B. E-G. Longitudinal section of apical region of a 35 mm. long root. E, potassium, F, starch: horizontal shading. Sugar, dotted. G, protein.

Starch was absent from all tissues of the young root, with the exception of the root-cap and endodermis (Fig. 3, B, D, F). But in older roots it tended to accumulate in the cortical parenchyma in the basal region, e.g. in an 85 mm. root it extended upwards for 20 mm. from the base.

Sugar was found throughout the root except in the meristematic tip; within the apical 5 mm. the last remaining traces of precipitate were found as small round granules in the tracheides of the protoxylem (Fig. 3, F).

The stem.

In the series of stems examined, ranging from very young to old and yellowing stems, the type of distribution exhibited by potassium remained remarkably consistent throughout the season. A complete description of one stem will therefore be sufficient to show the general distribution of the element. An exception is made, however, in the case of the etiolated plants, which are dealt with in a section by themselves.

The normally grown plants showed no sign of potassium starvation, and in the stem the only cells without some trace of the element were dead cork cells.

TABLE I.

Lengths of Apical Regions showing Richness in Potassium and Protein.

Region.	Description.	Potassium. mm. from tip.	Protein. mm. from tip.
Root-tip	Sprout, primary root . {	2.745	2.27
		2.70	2.40
		2.49	2.26
	Sprout, secondary root {	1.17	1.08
		1.08	0.972
		1.044	0.97
Shoot apex	Seedling radicle . . {	1.35	1.20
		1.23	1.05
		1.05	0.975
	Sprout . . . {	2.73	2.475
		3.0	2.70
	Etiolated shoot . . {	2.25	2.1
		2.40	2.25

Plants raised in sand from whole tubers and from small pieces with only one 'eye' showed the characteristic distribution of potassium, with only slight variation of intensity, although the growth, especially of the plants grown from pieces of tuber, was much less vigorous.

When sections of the stem apex, after appropriate treatment, were examined by the naked eye, it could be seen that in the apical bud of very young, closely arranged leaves, unseparated by internodes, potassium and protein were most densely located. In this region the majority of the cells both of leaves and axis were small and cubical, and had very abundant contents, being as yet incompletely vacuolated.

In the stem below the apex, and in all lower internodes, potassium and protein were concentrated in two cylinders of cells—several layers deep—one in the outer cortex, the other coinciding with the vascular cylinder, leaving the centre of the stem and the zone between the two cylinders relatively poor in these materials; the parenchymatous cells of these latter zones were vacuolated, so that their contents were in any case rather scanty.

At the nodes potassium was locally concentrated round the base of outgoing members.

Under the microscope it could be seen that the cells of the stem apex, i.e. meristematic region and rudimentary leaves, contained potassium in sufficient quantity to produce a dense black precipitate. The first region

to show in its cells distinctly less precipitate than the rest was the medulla, followed a few cells farther down by the inner cortex. The dense protein region at the apex did not extend quite so far down the axis, and the thinning out in the inner cortex and medulla became apparent before any lessening in the density of the potassium precipitate could be observed.

Sugar was not found in the apical 5 mm. of the stem; a scanty but widespread precipitate was given, however, with Fehling's at 1 cm. behind the apex; below this the precipitate increased rapidly to a maximum beyond which it was impossible to judge if more were present.

Starch, as already stated, was present in the inner cortex and medulla, just behind the apex of the sprout, but it was only a temporary deposit and no longer appeared in this region in the young growing stem. Even later, when the leaves were fully grown, starch was deposited only in the inner cortex and medulla of the lower nodes of the main stem and in the lower laterals on which tubers were being formed.

By careful comparison with normally grown plants it seemed possible to recognize a slight reduction both in intensity and extent of the potassium precipitate in sections from plants grown in sand. The most obvious explanation was that these plants were suffering from incipient potassium starvation, but it was not possible to draw any definite conclusion without the confirmation of quantitative analysis.

The etiolated stem.

The etiolated plants were grown in a specially constructed dark box in the laboratory greenhouse, and in pots 9 in. deep. Altogether eighteen plants were raised, of which twelve were from whole tubers, six were in sand, and six in sawdust. The remaining six were raised from small pieces of tuber with one 'eye' each—three in sand and three in sawdust.

All the plants grown from whole tubers produced remarkably strong white shoots, averaging about half an inch in thickness and 20 to 30 in. in length. They showed almost complete etiolation.

The plants grown from pieces of tuber were obviously smaller than the others, being only 6 to 12 in. long and rarely exceeding a quarter of an inch in diameter. Those grown in sand especially showed signs of starvation.

In these etiolated plants the distribution of potassium, protein, starch, and sugar was, with a few exceptions, similar to that already described for a normal plant grown in the light. Only the variations from this type will therefore be given in the following account.

(a) Plants grown from whole tubers.

One of the most obvious differences was the presence of starch in the region 5 mm. to 1 cm. down from the short apex, and there were marked

aggregations of this material round the axillary members of the nodes, where potassium and protein also were more concentrated than in the normal stem.

Sugar was absent from the apical 5 mm., but present in the remainder of the shoot; the amount of sugar per cell seemed to diminish slightly in the outer cortex and to be aggregated in the medulla.

Scale-leaves on these etiolated shoots contained abundant potassium and protein, especially in the hairs. Sugar was not present in the hairs, and starch only occurred in the guard-cells.

In older stems and their laterals the starch reserves were quite considerable. Protein showed signs of thinning out, giving a much less strongly marked colour reaction in the middle, as compared with the upper and lower internodes. There was no corresponding diminution in the quantity of the potassium precipitate; evidently ability to form protein was a factor tending to limit growth.

(b) Plants grown in sand from pieces of tuber.

In these plants, while the potassium precipitate seemed, if anything, slightly greater and more uniformly distributed than in the tissues of a normal plant, the protein material appeared very scanty indeed; the young laterals contained more than the middle and lower internodes of the main stem—as if in starvation the protein travels to the younger laterals at the expense of the older regions of the main shoot. Another sign of starvation was the almost complete absence of starch from these stems.

Compared with a plant also grown from a piece of tuber in sand, but in normal light supply, it appears that whereas in the dark there are signs of protein formation being a limiting factor, in the light growth continues until potassium or other minerals become limiting.

Roots.

The roots were not investigated as systematically as the stems, but from time to time those that were examined showed considerable uniformity, and very little essential difference from the young roots already described in detail in the section on the sprout.

The meristematic cap and vascular cylinder contained by far the most abundant precipitation of potassium and protein. The aggregation of these materials in the outer cortex, which was obvious in the younger piliferous and elongating regions, was less discernible in the older regions; in fact, towards the base of the root the total potassium in the cortical regions became very scanty indeed, though it was never entirely absent. Small secondary laterals produced in this older region of the main root were, however, abundantly charged with potassium, especially in the vascular elements.

In a large, secondarily thickened root of pentarch structure the phloem

contained much potassium, but the secondary xylem, especially the vessels and the ray-cells, contained very little.

Starch was present abundantly in the inner cortex, in all but the younger growing regions of the root. Sugar was only absent from the meristematic apex where protein and potassium were most densely located.

The green leaf.

The following description of the distribution of potassium, protein, starch, and sugar is typical of what was found at any stage of the active life of the leaf.

In the blade (Fig. 4, A and C) potassium was abundant in the chlorophyll-containing cells, the precipitate being greater in the palisade than in the spongy mesophyll. The chloroplasts were, however, entirely free from potassium. Frequently very large granules of the potassium precipitate were seen to be localized at the tops of the long palisade cells, just below the epidermis. Of the remaining tissues, the hairs, stomatal guard-cells, and vascular strands contained considerable potassium precipitate, the epidermal cells comparatively little, and the large parenchymatous cells surrounding the bundle in the midrib and larger lateral veins scarcely any at all.

All the tissues of the leaf contained abundant protein, but it was especially dense in the palisade, on account of the depth of staining of the chloroplasts, in the phloem, and in the epidermis.

Sugar was present in all cells. Starch occurred most abundantly in the mesophyll cells—both in the cytoplasm and inside the chloroplasts as little rod-shaped granules; it was also present in the guard-cells and in the starch sheath on the under side of the midrib. The amount of starch in the leaf varied, as was to be expected, with the time of day at which the leaves were examined.

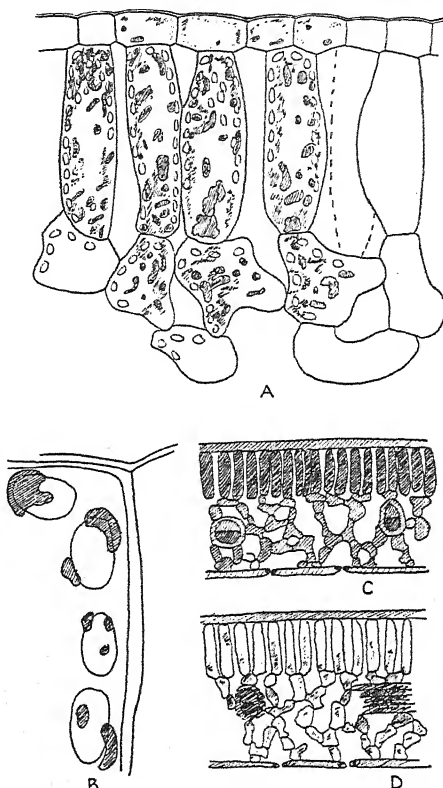


FIG. 4. A-D. Potassium in the leaf. A. Transverse section of blade showing potassium precipitate—shaded—in the cells of palisade and spongy mesophyll. B. potassium granules adhering to the surface of chloroplasts. C, diagrammatic representation of potassium distribution in a green leaf, and D in a yellowing leaf.

The petiole: the vascular strands, and one or two of the outermost layers which contained chlorophyll were densely filled with protein and potassium; the intermediate cells contained a certain amount, but being large and parenchymatous like those in the mid-rib of the blade, they did not look very full. Starch was only present in the chlorophyll-containing cells, and in the starch sheath below the bundle. Sugar occurred, but seemed to be less abundant than in the blade.

The yellowing leaf.

In a yellow leaf the mesophyll cells contained scarcely any potassium (Fig. 4, D); no starch was formed in them, and protein was no longer densely located in the palisade cells.

A migration of potassium from the leaf into the stem began while the leaf was still green but past maturity, because in the oldest green leaves a marked diminution in the potassium content of palisade and spongy mesophyll cells farthest away from the bundle, and a greater concentration of it in the cells of the conducting elements could be observed. Then in the next oldest leaves, which had turned yellow, the potassium had migrated almost completely from the mesophyll into the veins. Traces of the precipitate remained longest in the epidermal cells (cf. Fig. 4, C and D).

The flower.

All sections of flower bud and flower revealed abundant contents of potassium (Fig. 5, A) and protein, the greatest concentrations being in the anthers and ovules. Sugar was present, but no conclusion could be drawn as to its exact localization; starch appeared to be entirely absent.

The flower axis was richer in potassium than the internode below it, but the sepals and petals of an open flower contained less than the young foliage leaves. Chlorophyll occurred in the outer layers of the sepals where potassium and protein were also more concentrated.

In the anther (Fig. 5, B) potassium was very abundant, except in the tapetal layer, from which it was absent, having probably been drained therefrom to feed the pollen grains. Some pollen grains contained potassium, others appeared to be without it (Fig. 5, D and E); this may have been due to non-penetration of the reagent through the thick wall, or perhaps a result of degeneration on account of sterility. Evidence of sterility in the stamens of the species of potato examined is seen in the poor development of sporogenous tissue—the pollen sacs, in transverse section, appear as narrow horseshoe-shaped cavities instead of the ordinary spherical type (Fig. 5, B).

In the ovary (Fig. 6, A) potassium was quite abundant in the wall and placental regions, but was most concentrated in the ovules and the tissues of the placenta adjacent to them.

The young fruit. In specimens not quite 1 cm. across the widest diameter, the placenta and ovules had not increased much in size, but the ovary wall had made considerable growth, so that there was a large cavity

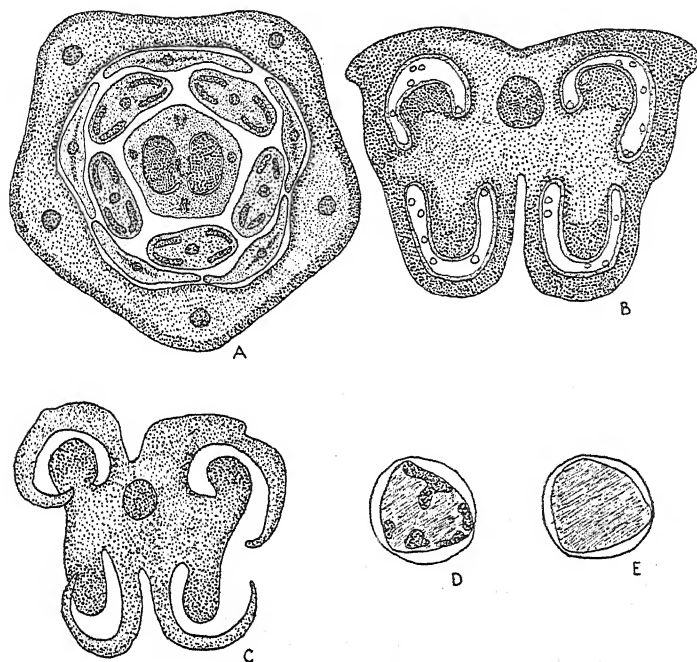


FIG. 5. A-E. Distribution of potassium in the flower. A. Transverse section of very young flower bud showing regions where potassium is most densely localized—deep shading. B and C. Transverse section of anther: localization of potassium in the young (B) and old (C) stage. D-E, potassium in pollen grains. In D the granules are clearly defined. E, a grain which shows no precipitate.

in each loculus. Potassium showed the same distribution here as in the unfertilized ovary, but appeared to be more abundant. It was slightly more concentrated in the outer green layers of the pericarp wall, and the middle layers traversed by vascular strands (Fig. 6, B).

The older fruit. In specimens examined, varying from 2.5 to 4 cm. in diameter, it was found that potassium had accumulated in the tissues, all of which contained abundant precipitate: the outer layers of the pericarp, the vascular strands, and the outer layers of the young seeds relatively the most, the endosperm and embryo of the seeds relatively the least (Fig. 6, C). Considerable quantities of starch were stored in the large parenchymatous cells of the inner pericarp and the greatly swollen placentas. Protein, except for a relatively greater abundance in the endosperm and embryo, showed essentially the same distribution as potassium.

The seed. Owing to its small size and the fact that the embryo is

coiled inside the endosperm, it was very difficult to obtain sections of the ripe seed which would show the distribution of potassium. However, it was found that the outer layers of thickened cells of the testa contained a

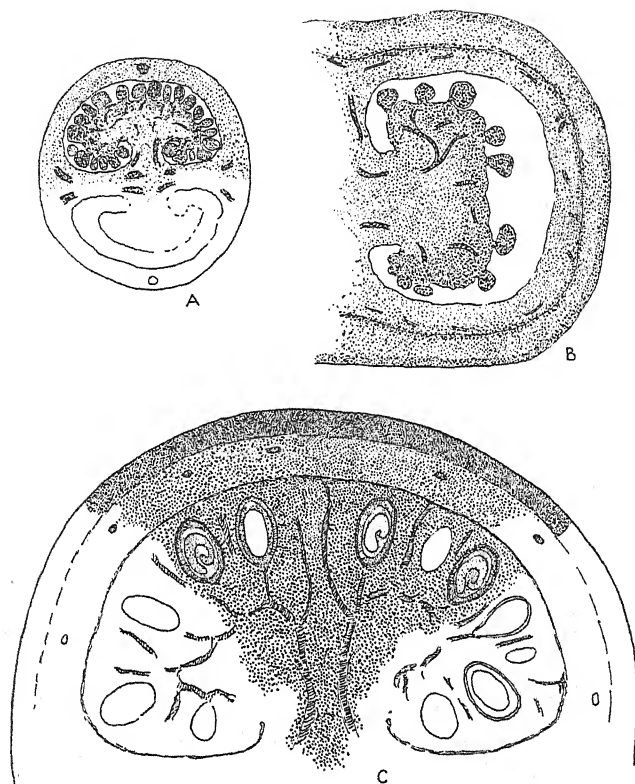


FIG. 6. Distribution of potassium in the ovary and fruit. A. Transverse section of ovary before fertilization. B. Transverse section of young fruit. C. Transverse section of older fruit.

little potassium, the inner dead crushed layers more. The endosperm and embryo contained relatively more potassium than in the unripe stage described above, the precipitate being more abundant in the endosperm (Fig. 7, A). On the other hand, the embryo appeared to be richer in protein than the endosperm (Fig. 7, B). Starch and sugar were absent at this stage, but oil globules were present in all cells except those of the testa.

Germination of the seedling.

The seedlings are very small, and it was not found possible to test very young seedlings with more than one reagent. The cotyledons are epigeal, the hypocotyl generally commencing to elongate when the radicle is about 8–12 mm. long. By the time the cotyledons have unfolded the initial food supply is exhausted and the seedlings cannot develop further

unless both root and cotyledon are able to function. This was seen in the case of seedlings grown in sand and etiolated seedlings, which began to die off at this stage, the one set because the roots could not obtain further salt supply, the other because the cotyledons could not assimilate.

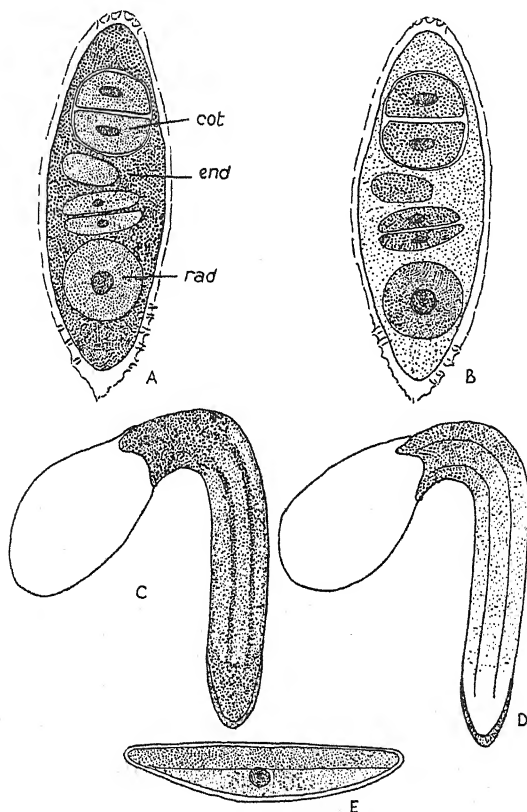


FIG. 7. Distribution of potassium, protein, starch, and sugar in the seed and seedling. A. Longitudinal section of seed: potassium. B. protein. The coiled embryo is cut in transverse section (*cot.*, cotyledon, *rad.*, radicle, *end.*, endosperm). C and D, very young seedlings before cotyledons have emerged. C, potassium, D, starch—deep shading, sugar—light shading. E. Transverse section of cotyledon: potassium.

The type of distribution of potassium, protein, starch, and sugar in the young radicle was on the whole the same as in the adventitious roots of the sprouts derived from tubers. In the hypocotyl end of the root just where it emerged from the seed there was a noticeable localization of protein, potassium, and starch (Fig. 7, C and D). This would appear to be a temporary accumulation, because it disappeared as the cotyledons emerged and the stem axis commenced to grow. The distribution in the young stem was essentially the same as in a stem belonging to a sprout from a tuber, and need not be described in detail again.

The deep green coloured cotyledons only differed from the other leaves in that the differentiation between palisade and spongy mesophyll is slight, hence the distribution of potassium and protein in the mesophyll was more uniform (Fig. 7, E).

Tests were made on seedlings of different ages, but in all essentials the distribution of potassium, protein, starch, and sugar in the root, stem, and leaf of the seedling was found to be exactly as previously described for the tuber plants grown from tubers.

DISCUSSION.

The purpose of the present investigation was chiefly to determine the distribution of potassium inside the potato plant during a growing season, with a view to narrowing the problem of its functions. The results here given represent what has been determined microchemically.¹ They are based on differences observed in individual sections, which differences were consistent for all the sections of any one tissue. As the sections were on the whole of uniform thickness, differences of density of the precipitate within any one section can legitimately be taken to indicate differences in the quantity of potassium in the tissues. The general conclusion is that regions of special physiological activity contain potassium in considerable quantity. The unvarying presence of potassium in considerable quantity in all actively dividing tissues, such as primary and secondary meristems and reproductive tissues, adds confirmatory support to the already strong belief (Reed (16), de Vries (19), Gregory and Richards (8), Janssen and Bartholomew (7), James (6)) that plentiful supplies of potassium are essential for growth and cell-division. Some of these authors have based their conclusions on the movement of potassium in plants starved of the element. In all cases it was found that potassium tended to move out of the older regions, especially old leaves, and to be translocated to the young leaves and meristems where its presence apparently led to further growth. In the potato plant, for example, it was found that potassium was re-absorbed into the stem from the older leaves as they began to fade. The migration in this case, however, might probably be related to age rather than to a deficiency of potassium, as no other sign of starvation was evident and there was no lack of potassium in the internodes of the stem above and below the yellowing leaf.

That potassium is closely related to the process of photosynthesis seems obvious from its distribution in the leaves while they are active and green, and its withdrawal, at least from the mesophyll cells, during yellowing. The positive effect of potassium fertilizers upon assimilation and

¹ Quantitative data have been obtained for certain regions of the plant and will be given in a subsequent paper.

carbohydrate production is shown by a number of workers, e.g. Russell (17), Gregory and Richards (3), Maskell (11), Schertz (18), Janssen and Bartholomew (7), Remy and Liesegang (15), James (5).

Potassium was not found in the chloroplasts of any mesophyll cells, cf. Macallum (9), Weevers (22), and Lloyd (8). It was found, however, very closely associated with chloroplasts (Fig. 4, B). Lloyd points out that in the cell the distribution of the precipitated potassium does not necessarily represent the actual distribution in the living condition; it is not impossible therefore that potassium was present inside the chloroplast and nucleus in the living state, but that precipitation took place on the membrane owing to the diffusion of the ion towards the entering reagent. Even if potassium was not present in the chloroplasts in life, the fact that it appeared closely associated with the chloroplast surface, at which photosynthesis probably occurs, is significant.

In some of the older leaves examined, which had not shown a change from green to yellow, evidence of potassium migration from the palisade cells was observed. It appears, therefore, as if the withdrawal of potassium might be a causal factor in yellowing, rather than a result; and that the functioning of the chloroplasts actually depends upon its presence. James (5) suggests that the loss of potassium is a causal factor in the ageing of the leaf, and Russell (17) gives abnormal colour of leaves as one of the signs of potassium starvation. On the other hand, the effect of potassium fertilizers on chlorophyll formation in the potato has been investigated by Remy and Liesegang (15), who report that potassium deficient plants contain more chlorophyll per unit of leaf mass than do plants well supplied with potassium. Schertz (18) also states that in potatoes abundant potash suppresses pigment formation in the chloroplasts. The evidence seems, therefore, to be rather conflicting, and it is obvious that more work is necessary before the relationship, if any, between potassium and chlorophyll can be defined.

The careful and comprehensive work of Mason and Maskell (12-13) has clarified the present position of research on translocation in plants. These workers show that carbohydrates are transported in the phloem; that inorganic nitrogen absorbed by the roots is conducted by the xylem, probably in the transpiration stream, to the leaves; and that elaborated nitrogen compounds such as proteins together with the carbohydrates are translocated in the phloem. In the potato plant protein and potassium were very abundant in the phloem of all organs. The constant appearance of potassium in considerable quantity in a tissue concerned in translocation of organic substance suggests that it may be a factor in the process.

The movement of potassium itself is of interest. It was shown in the second paper of this series (6) that the downward movement of potassium is probably independent of the transpiration stream, and the presence of

considerable quantities in the phloem suggests that this tissue may be the path of conduction. Ringing experiments by Mason and Maskell (14) point to the same conclusion.

Perhaps the most interesting result of this work on the potato is the strong evidence obtained of a close similarity between the distribution of potassium and of protein; the physiological association of these two has been suggested before, but no very strong support advanced in favour of it. The close association of potassium and protein in meristematic regions, where protoplasm is being actively synthesized, and again in such tissues as the outer cortex, phloem, palisade, and protein storage tracts of the tuber is very clearly indicative that potassium is a factor in protein metabolism. Therefore an investigation into the nature of the relationship between protein and potassium might prove a very profitable one. The most obvious suggestion is that potassium may be necessary in promoting the activity or formation of proteolytic enzymes. Hartt (4) actually found in the sugar-cane that potassium deficiency decreased the peptase activity of blades, sheath, stem, and roots, and Janssen and Bartholomew (7) showed that in tomato plants potassium deficiency prevented synthesis of organic nitrogenous substances. Both these results support the view that the presence of potassium is a factor in protein synthesis.

SUMMARY.

An investigation—employing microchemical methods—into the distribution of potassium and associated organic materials in the tissues of the potato plant at progressive stages of its growth is described.

All cells, except dead cork cells, contain some potassium precipitate, which is localized only in the cytoplasm and vacuole, and not in the nucleus and plastids.

The tissues which contain most potassium, as estimated by the density of the cobalt hexanitrite precipitate in their cells, are: (*a*) the apical meristems of roots and shoots, (*b*) the outer regions of the cortex, especially in stems, and to a less extent in roots, (*c*) the phloem, (*d*) active green leaves, (*e*) reproductive organs.

The type of localization found in the stem and root remains fairly constant throughout the growth period examined.

In the early stages of development the leaves have a very dense potassium precipitate in all their green cells, particularly in the palisade. As the leaf increases in size the density of the precipitate, although still great, appears to be slightly less than before; this may possibly be explained by the enlargement of the individual cells by vacuolation tending to decrease the observed density.

In the yellow leaf, the cells of the mesophyll, particularly the palisade,

lose their potassium ; the chloroplasts show signs of disintegration ; while the elements of the vascular bundles are full of potassium not yet conducted away.

During tuber formation an increase of potassium precipitate beyond that of the normal stem is observed in the swollen tip region.

Comparing potassium with organic materials an almost exactly similar distribution is shown by protein. Starch is stored in parenchymatous cells of the stem, inner cortex, and pith, where potassium and protein are relatively scanty. But in the tuber potassium and protein are abundant also in starch-storing cells.

The conclusions, some of which are in agreement with previous views, are that regions of special physiological activity contain potassium in considerable quantity, and particularly is this so in the regions concerned with (*a*) meristematic division, (*b*) photosynthesis, (*c*) translocation and storage of food materials.

The close association of potassium with protein in nearly all the tissues also suggests that it may be necessary for protein metabolism.

ACKNOWLEDGEMENT.

The writer wishes to take this opportunity to thank Professor A. G. Tansley, in whose department this work was carried out, for his unfailing kindness ; and Dr. W. O. James, who suggested and supervised the research, for his most helpful advice and criticism.

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The Estimation of Carbon in Plant Tissues.

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With two Figures in the Text.

DURING the course of research into the metabolism of the coffee plant it was found necessary to estimate total carbon, and carbon in the form of sugars and starches, in plant tissues. A large number of samples were involved, and search through the literature failed to reveal any technique sufficiently rapid and accurate to serve the purposes of our work. Because of this the methods outlined below were developed: these would, normally, have been published with the results of our researches, but as several inquiries have been received from other workers it has been decided to publish a short preliminary account of the methods in use here.

It should be emphasized that there is nothing inherently new in the principles of these. Ashby, Bolas, and Henderson (1) used a gas-analysis method of estimating carbon in *Lemna* fronds, while the sugar estimation is a development of the micro-method of Hagedorn and Jensen (2). Nevertheless, we believe that a useful purpose will be served by the publication of such an account. The writers have spent some time testing various methods of analysis and have come to the conclusion that the methods here advocated are sufficiently rapid, accurate, and easy to be generally adopted for biological inquiries involving carbon estimation.

A. Determination of Total Carbon.

The basis of the method employed consists of burning the material in a current of oxygen, passing the gases through heated copper oxide, as in an ordinary combustion determination, and collecting them in a Haldane type burette. The carbon is determined by measuring the difference in volume before and after absorbing the carbon dioxide by potash.

The most important part of the apparatus is the design of the

combustion tube, enabling the material to be introduced into a hot furnace and thus obviating heating delays.

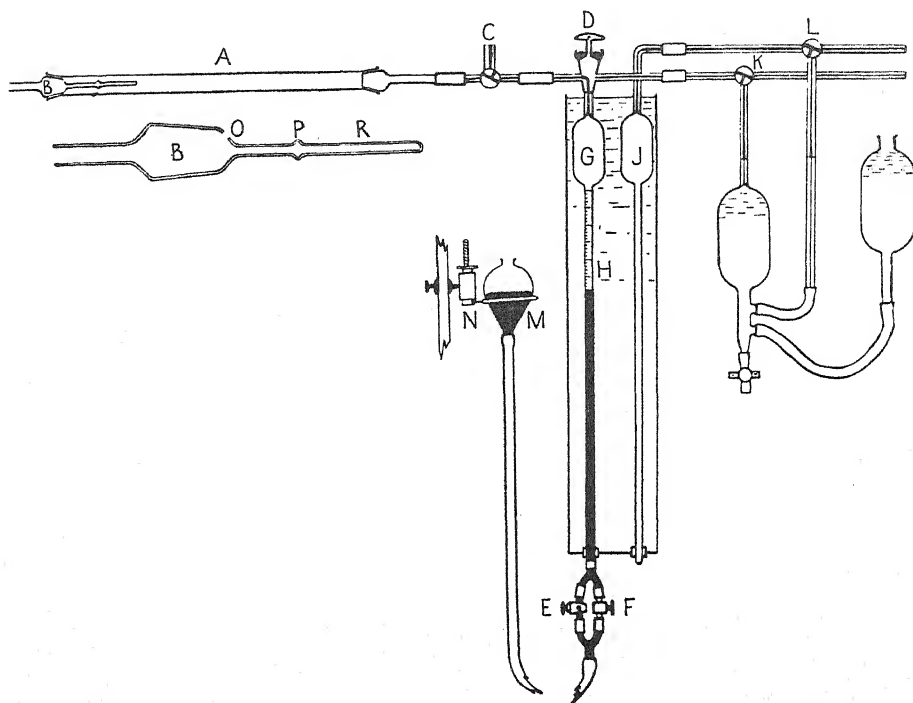


FIG. 1. Diagrammatic sketch of the combustion apparatus.

The apparatus will first be described in detail and then a description of the method of carrying out the determination will be given.

(a) *The combustion tube.*

This consists of a transparent silica tube (A, in Fig. 1) with ground-in stoppers at both ends: the tube being 50 cm. long and 1 cm. wide.

The stopper (B) at the entrance to the tube is of special design, so as to enable the material to be introduced into the red-hot tube and thus effect a considerable saving of time. A rod, R, 8 cm. long and 6 mm. diameter is joined to the inner end of this stopper. The oxygen is admitted through a small hole, O, 1 mm. across, in the end of the stopper. In the centre of this rod is a ridge, P, 8 mm. in diameter.

The use of this stopper is as follows:—An oxidized copper gauze spiral is fitted on the end of the rod, R, extending from the ridge, P, to the end: the combustion tube is not heated beyond the point reached by the ridge, P. In introducing the plant material, which consists of about 10 mg. in a small platinum boat, the stopper is removed, the boat placed

in the entrance of the tube, and the stopper pushed rapidly into place, the rod, R, thus pushing the boat into the heated part of the tube. The reason

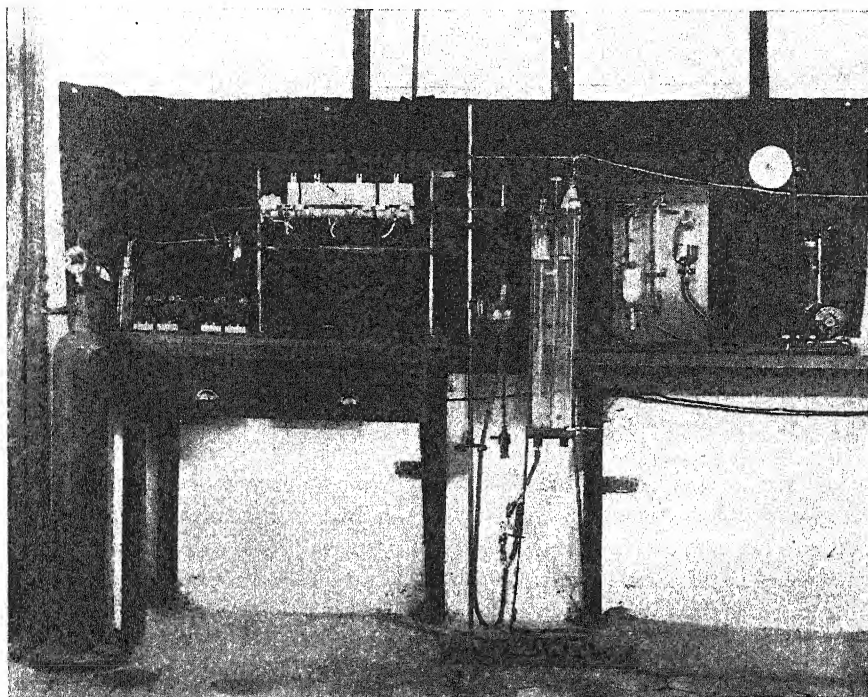


FIG. 2. Photograph showing the combustion apparatus complete.

for the position of the hole, O, is to avoid any dead end to the tube in which gases might collect and be difficult to wash out, and also so that the copper-oxide gauze is in the oxygen stream.

The stopper at the other end is an ordinary one and calls for no comment.

(b) *The combustion furnace.*

A specially designed electrically heated furnace is employed, consisting of four separately wound and separately adjustable sections. These sections are mounted on horizontal bars so as to be movable from side to side; they are also hinged in the middle so that they can be opened up to allow the combustion tube to be easily inspected.

The lengths of these sections are $1\frac{1}{2}$ in., 1 in., 8 in., and $3\frac{1}{2}$ in., to correspond with the fillings of the tube. The whole furnace is mounted on two vertical rods to allow for adjustment up or down.

(c) *Gas-measuring apparatus.*

This consists of a Haldane type burette designed to suit the special needs of the apparatus.

The bulb, G, is of approximately 85 c.c. capacity and the tube, H, 15 c.c.; this tube being carefully calibrated into 0.1 c.c., the divisions being sufficiently far apart to allow of estimation to 0.01 c.c. The balance tube, J, is of the same shape and capacity. At the top of the burette is a mercury-sealed four-way tap, D, by means of which the burette can be put into communication with either (a) the air, (b) the combustion tube, or (c) the absorption apparatus.

A second stopcock, C, an ordinary three-way one with capillary limbs, connects, by means of ground-in joints, the combustion tube and the gas burette.

The gas burette and balance tubes are immersed in a copper thermostat, with glass in the front and back, and provided with an efficient stirring device.

The lower end of the gas burette protrudes through the bottom of the tank and connects with a mercury reservoir, M. Between the reservoir and the gas burette are a glass stopcock, E, and a steel needle valve, F, connected by Y-tubes so that either or both can be used to pass the mercury. The stopcock, E, allows the burette to be rapidly filled with mercury; it is then closed and the combustion gases can be drawn slowly into the burette by adjusting the needle valve, F, for a slow rate of fall of the mercury.

A retort stand with three open rings clamped on it is used to support the mercury reservoir. The rings are so placed that with the reservoir in the top one mercury just fills the burette; in the bottom one it falls to about the 15 c.c. mark. The centre ring has a fine adjustment to allow of accurate levelling.

(d) *The absorption apparatus.*

This consists of an ordinary potash absorption apparatus of such a capacity as to take the whole contents of the gas burette at once.

Exact levelling can best be done by the aid of two miniature burette readers of the Hyman's type, attached to the capillary with bent paper clips.

(e) *The oxygen supply.*

A cylinder of oxygen fitted with a fine adjustment valve is used and in the initial determinations this together with the needle valve, F, on the gas burette were used to adjust the regular flow of oxygen. As, however, it is difficult to adjust the two to the same extent, a large Woulff's bottle, of 15 litres capacity, is placed between the cylinder and the combustion

tube, and is fitted with a mercury trap at the centre tubulure so that oxygen escapes when the pressure exceeds 1 in. of mercury. The cylinder valve is adjusted so that oxygen is escaping slowly at this trap all the time: constant pressure is thus maintained in the apparatus. A caustic soda wash bottle is placed in between to remove any carbon dioxide.

PROCEDURE.

The combustion tube is filled as follows:—After washing well with chromic acid-sulphuric acid mixture, it is rinsed with distilled water and dried by warm dry air.

Lead peroxide asbestos is prepared as described by Pregl (3), and a layer 3 in. long is placed, between two wads of asbestos, at the far end of the tube.

Next to this wire-form copper oxide, which has been impregnated with about 25 per cent. of lead chromate, is poured into a length of 8 in., followed by an asbestos plug to keep it in place. The rest of the tube remains empty. Copper gauze is wound around the end of the rod on the entrance stopper, so that it will just slide easily into the tube, and it is then oxidized ready for use.

The tube is placed in the furnace so that the 8 in. length of copper oxide and the $3\frac{1}{2}$ in. length of lead peroxide asbestos are in the corresponding length sections of the furnace: the $1\frac{1}{2}$ in. furnace section is over the copper-oxide gauze.

The fillings must be so placed that when the four furnace sections are just touching, and when the end one (the $1\frac{1}{2}$ in. section) is over the gauze, then the tube in the second section (1 in.) is empty and the layer of copper oxide commences at the beginning of the 8 in. section. A slow stream of oxygen, about two or three bubbles a second, is then passed, and the furnace turned on and regulated so that the first three sections heat up to a bright red heat and the last section, around the lead peroxide asbestos, to 200° C. (the lead peroxide being destroyed at temperatures much above this). After heating in this manner for about three hours the apparatus is ready for use.

The apparatus is to be employed by the authors for the determination of carbon in dried plant tissues, but preliminary work on pure organic compounds has shown that accurate results can be obtained for almost any substance if suitable precautions are taken.

The method is designed primarily for rapidity and such rapidity is to a large extent possible owing to the nature of the material, which allows of it being placed directly into the red-hot combustion tube, thus saving twenty minutes or more in heating up. It is obvious that if a volatile substance be used it must be inserted in the cold. The following description

of the actual determination must therefore be understood to be applicable only to dried plant tissue or other not readily volatile substances (the necessary modifications for such substances being given later).

Enough of the substance to yield between 10 c.c. and 15 c.c. of carbon dioxide, on combustion, is carefully weighed out into a very small platinum boat (weighing about 0.2 grm.), the weights being measured to 0.000005 grm. and recorded to 0.00001 grm. using a Bunge microbalance. Although dried plant material is fairly hygroscopic it was found to be possible to weigh so rapidly on this balance, once the worker was accustomed to the amount of material to take, that a weighing bottle could be dispensed with, since the increase in weight due to absorption of moisture was less than 0.000005 grm. in the time taken (1 to 1.5 minutes).

The current to the combustion furnace is switched on, and whilst it is heating up the mercury reservoir is raised so as to fill the burette, the stopcock, E, being opened to facilitate this. Stopcocks C and D are then turned so as to connect the burette with the combustion tube, stopcock E is closed, the reservoir placed on the bottom ring of the stand, and the needle valve, F, adjusted so that the mercury running out of the burette draws oxygen through the combustion tube at the rate of two to three bubbles a second, as shown by a small sulphuric acid bubbler.

When the furnace is ready the burette is filled with mercury to the base of the stopcock, which is then turned so as to cut off the burette altogether, the mercury reservoir is lowered to the bottom ring, and stopcock C turned so as to connect the combustion tube with the air. The stopper B is now opened, the platinum boat placed just in the mouth of the tube, and the stopper at once replaced and pushed well home, thus pushing the boat into the heated part of the furnace (into the second section). A slight pressure is at once set up in the tube, oxygen ceasing to go through the bubbler, owing partly to the heating up of introduced cold air and partly to the combustion of the material in the boat. If the tube has been correctly filled this pressure is reduced in the course of about half a minute by oxygen being driven out into the air through the stopcock, C, and as soon as the stream of oxygen begins to pass through the bubbler again the cocks C and D are at once turned to connect the combustion tube to the gas burette. This process was found necessary so as to prevent any of the gases being driven out of the front end of the combustion tube by the increased pressure: experiment showed that no errors were introduced by this momentary opening to the air.

The stream of oxygen is allowed to pass at the rate of two to three bubbles a second until the burette is about a third filled, when the rate is slowly increased, by adjusting the needle valve, so that the burette and graduated tube are filled in about five minutes. When only 2 or 3 c.c. of the tube are left to be filled the stopcock E is opened for the final adjustment.

When the mercury is almost at the bottom of the graduations the stopcocks C and D are turned into their original positions. The mercury is then levelled by eye and the absorption apparatus also levelled. The stopcock D is now turned to connect the burette and the absorption apparatus, stopcocks K and L being open to the air, and the first reading of the burette is taken. K and L are turned to disconnect with the air and the gas mixture passed into the potash bulb and back again to the burette four times, by raising and lowering the reservoir. The absorption apparatus is then relevelled by means of the fine adjustment ring, the contraction in volume read off, and the volume of carbon dioxide at N.T.P. calculated. The thermostat tank is not regulated to any special temperature but is provided with an accurate thermometer.

As soon as the one determination is completed the burette can be refilled with mercury and a second determination commenced at once.

When using somewhat volatile substances it is found necessary to open out the two front sections of the furnace on their hinges so as to allow the tube to cool slightly: if very volatile substances are employed they must be inserted with these two sections cold. In either case the manipulation of the two stopcocks C and D is not now necessary, the burette being connected directly to the combustion tube and the stream of oxygen being slowed down to about a bubble a second until combustion of the substance begins. Otherwise the procedure is the same as before.

Accuracy of method.

The smallest volume of carbon dioxide that can be measured on this apparatus is 0.01 c.c., which is about 0.008 c.c. at N.T.P. (the atmospheric pressure in these laboratories being about 680 mm.). This volume is equivalent to 0.000043 gm. of carbon, which is a much smaller amount than can be determined by the ordinary combustion method. Experiment has shown that duplicate readings never differ by more than 0.01 c.c. So that even allowing for an error of a full 0.01 c.c. in the reading there will be an error of less than 0.00005 gm. in the weight of carbon determined.

In weighing the substance to be employed about 10 mg. are taken and recorded to the nearest 0.00001 gm. (weighing being conducted actually to 0.00005 gm.), so that in both the original weight and the carbon determined the errors do not exceed 0.00001 gm., and the percentage of carbon determined should thus be correct to the nearest 0.05 per cent. of substance taken or 0.1 per cent. of the actual carbon content for plant tissue containing 50 per cent. carbon.

It will be seen that if the burette is graduated in smaller divisions then greater accuracy can be obtained. For the purposes of the investigation in hand, results correct to 0.1 per cent. are sufficient.

In order to test the apparatus combustions were carried out on several

pure organic compounds, mostly containing nitrogen, and the results are shown in Table I.

TABLE I.

Substance.	Weight taken.	Carbon found.	Theoretical C.
	gram.	%.	%.
Dextrose	0.01001	40.0	40.0
	0.00998	40.0	
	0.01141	40.1	
Acetanilide	0.00808	71.1	71.1
	0.00701	71.2	
Dimethylglyoxime	0.01004	41.3	41.35
	0.01072	41.4	
Urea	0.02344	20.0	20.0
	0.02440	20.0	
	0.01511	20.0	
Hexamethylene-tetramine	0.02013	29.0	29.0

In Table II are given five results for the same sample of dried coffee leaf.

TABLE II.

Sample.	Weight taken.	Carbon found.	Mean.
	gram.	%.	%.
Coffee Leaf No. 4	0.00950	48.3	48.35
	0.01234	48.4	
	0.00844	48.3	
	0.00760	48.3	
	0.01370	48.4	

The figures in Tables I and II show that the method can be relied upon to give results consistent to 0.1 per cent.

Rate of combustion.

As one of the main considerations in introducing this method was to reduce the time of combustion, a series of determinations on coffee leaf was carried out, and it was found that if the combustion extended over five minutes correct results were obtained. Table III shows such a series.

TABLE III.

Time of combustion.	Carbon found.	Correct value.
min.	%.	%.
2	46.8	48.35
3½	47.8	
5	48.3	
5	48.4	

The actual time required for a complete determination, assuming the furnace to be heated up in advance, is about twelve to fifteen minutes, i.e. three minutes for weighing, five for the combustion, and about five for the absorption of carbon dioxide. It is thus possible to carry out a large number of determinations in a day.

Determination of hydrogen.

Although not originally designed for determining hydrogen, this apparatus can be readily so adapted by the insertion of an absorption tube between the combustion tube and the stopcock C.

In order to make the description of this apparatus complete a few determinations of hydrogen were carried out, using a calcium chloride absorption tube and reducing the time of combustion to ten minutes. If all the water has not been driven over into the absorption tube by the time that the gas burette is filled the cocks C and D are turned so as to close the burette and to open C to the air: whilst the carbon dioxide is being absorbed the remainder of the water may be driven over from the combustion tube to the absorption tube. The hydrogen is weighed, as usual, as water.

Table IV gives some results.

TABLE IV.

Substance.	Hydrogen.		Carbon.	
	Found.	Actual.	Found.	Actual.
	%.	%.	%.	%.
Urea	6.72	6.71	20.0	20.0
Hexamethylene-tetramine . . .	9.73	9.75	29.0	29.0
Phenolphthalein	4.42	4.43	—	—

B. Carbohydrate carbon.

Some time was spent in testing various methods for the estimation of sugars, with a view to selecting a rapid one which is both simple and at the same time accurate.

Most of the methods employed by other workers are based on the use of Fehling's solution with various modifications, but none of them seems to be wholly satisfactory, since careful standardization of the method is always essential, and this not only becomes irksome but also leads to frequent inexplicable errors.

A method described by Hagedorn and Jensen (2), for the determination of sugar in blood, was brought to the authors' notice: this method has been modified to suit the larger quantities of sugar encountered in the work in these laboratories and has given very satisfactory results.

The method is based on the fact that reducing sugars reduce potassium ferricyanide in alkaline solution to ferrocyanide: the excess of ferricyanide

is determined by adding potassium iodide and titrating the liberated iodine with sodium thiosulphate. Interference by the ferrocyanide produced is prevented by precipitating it with zinc sulphate.

Preparation of solutions.

British Drug Houses A. R. chemicals were used in all cases.

A. Weigh out exactly 3.20 gm. of pure dry potassium ferricyanide (dried in vacuum over sulphuric acid), dissolve in distilled water, and make up exactly to a litre. Stored in a dark bottle in the dark this solution keeps for several months.

B. Dissolve 21.2 gm. of pure anhydrous sodium carbonate in a litre of water.

C. Zinc sulphate—a 10 per cent. solution.

D. Potassium iodide—a 7.5 per cent. solution.

E. Acetic acid—a 3 per cent. solution.

F. Soluble starch—a 1 per cent. solution. This keeps quite well, but in practice a fresh supply was prepared weekly.

G. Standard sodium thiosulphate. Make up several litres of N/100, using well-boiled distilled water, to free it from carbon dioxide, and storing it in an aspirator fitted with a soda lime tube: in this way it keeps for some months. It should be standardized against potassium iodate each day of using; a potassium iodate solution of about 1 gm. of the carefully dried substance per litre being used—this keeps for months.

Procedure.

The estimations are carried out in 175 c.c. conical flasks.

5 c.c. of the sugar solution, containing not more than 0.003 gm. of reducing sugars, are carefully measured into the flask (if less than 5 c.c. of the sugar solution is taken the volume is made up with water). 10 c.c. of solution A, the ferricyanide solution, are then added from a carefully graduated pipette and 10 c.c. of solution B, the sodium carbonate, from an automatic pipette. (Much time and trouble can be saved by the use of automatically filling pipettes for all the solutions not requiring exact measurement, i.e. for all except the potassium ferricyanide.)

A blank is also prepared, using 5 c.c. of distilled water and 10 c.c. each of A and B.

The flasks are immersed in a bath of boiling water, the rings being placed around their necks so as to help keep them down, for exactly 20 minutes. They are then removed and cooled by placing in cold water for a few minutes.

After this add 15 c.c. of zinc sulphate, solution C (to precipitate the ferrocyanide formed), 10 c.c. of potassium iodide, solution D, and 25 c.c. of acetic acid, solution E. Titrate the liberated iodine with the N/100 thio-

sulphate solution, using three drops of the starch indicator when the yellow colour has nearly gone: the end point is very sharp.

It is convenient to mix together at the beginning of each day sufficient of solutions C and D, in the proportions of 15 to 10, for the day's work, and to add these together from a 25 c.c. automatic pipette to each flask. If mixed in bulk and stored iodine is slowly liberated, and this has to be removed before the solution can be used.

The difference in titration between the blank and the sugar solution gives the volume of thiosulphate equivalent to the ferricyanide reduced.

In practice a number of determinations are made at once, and it is not possible to do all the titrations at once, but experiment showed that no appreciable oxidation takes place in the flasks during 15 to 20 minutes, the average time required to titrate a series of 8 to 12 determinations. In the case of plant extracts it is usually necessary to do duplicates on each sample, and if these are titrated in the following order: B, 1, 2, 3, 4, 4, 3, 2, 1, B (where B stands for blank and 1, 2, 3, and 4 for the number of the sample), and the mean titration value for each sample taken, then any error due to the time of standing before titration should be eliminated.

In order to get consistent results it is necessary to use a burette graduated to 0.02 c.c. and to carry out the titrations to the nearest 0.01 c.c. This is readily done by using a fine tip, waxed on the outside to prevent creeping, so that one drop from it does not exceed 0.01 c.c. in volume. The end point is readily obtained to the nearest drop in clear solutions. A 10 c.c. burette is sufficient, provided that the thiosulphate solution is not weaker than about N/104.

Standardization.

The method was standardized against pure sugars (British Drug Houses A. R. chemicals), carefully dried in vacuum over sulphuric acid: dextrose was used for the main standardization and then sucrose, inverted, was checked against this.

(a) *Time of heating.* Immersion in the water bath for 15 minutes was first tried and, although consistent results could be obtained after this time if it were adhered to very strictly, it was found that 20 minutes was a much more convenient period, as a variation of about half a minute made no appreciable difference. Accordingly 20 minutes was taken as the time of heating.

(b) *Standardization against dextrose.* About 0.6 gm. of the carefully dried dextrose was weighed out accurately, dissolved in distilled water, and made up to a litre in a standard flask (with N. P. L. certificate). Different amounts from 0.5 c.c. up to about 5.0 c.c. were carefully measured out from a standard burette (with N. P. L. certificate) into the 175 c.c. conical flasks, and the determinations carried out as described above.

TABLE V.

Mg. Dextrose taken.	0.317	0.634	1.268	1.903	2.410	2.537	3.044
0.009207 N $\text{Na}_2\text{S}_2\text{O}_3$	1.02 c.c.	2.04	4.00	5.94	7.42	7.78	9.26
0.01 N $\text{Na}_2\text{S}_2\text{O}_3$	0.94 c.c.	1.88	3.68	5.46	6.84	7.16	8.52

Table VI gives the values as determined from the graph drawn with the above data.

TABLE VI.

Mg. Dextrose.											Difference. Add.								
$\text{Na}_2\text{S}_2\text{O}_8$	0.	1.	2.	3.	4.	5.	6.	7.	8.	9.	1.	2.	3.	4.	5.	6.	7.	8.	9.
0.	—	0.034	0.068	0.100	0.134	0.168	0.202	0.236	0.270	0.302	3	7	10	14	17	20	24	27	31
1.	0.336	0.370	0.404	0.438	0.472	0.506	0.540	0.574	0.608	0.642	3	7	10	14	17	20	24	27	31
2.	0.676	0.712	0.748	0.784	0.820	0.854	0.890	0.924	0.960	0.994	3	7	11	14	17	21	25	28	32
3.	1.030	1.066	1.100	1.134	1.170	1.206	1.240	1.276	1.312	1.348	4	7	11	14	18	21	25	28	32
4.	1.382	1.418	1.454	1.490	1.526	1.560	1.596	1.632	1.668	1.704	4	7	11	14	18	22	25	29	32
5.	1.740	1.776	1.812	1.848	1.884	1.920	1.956	1.994	2.030	2.066	4	7	11	14	18	22	26	29	32
6.	2.104	2.140	2.178	2.214	2.250	2.288	2.324	2.360	2.398	2.436	4	7	11	15	19	22	26	30	33
7.	2.472	2.510	2.548	2.584	2.622	2.660	2.698	2.736	2.772	2.810	4	8	11	15	19	23	26	30	33
8.	2.848	2.884	2.922	2.960	2.996	3.034	3.082	3.108	3.146	3.184	4	8	11	15	19	23	27	30	33
9.	3.222	3.260	3.298	3.336	3.374	3.412	—	—	—	—	4	8	12	15	19	23	27	30	33

(c) *Interpretation of results.* The number of cubic centimetres of N/100 thiosulphate equivalent to the ferricyanide reduced is calculated by subtracting the volume required for the sample under estimation from the volume required by the blank and multiplying this difference by the normality factor of the thiosulphate. For example, if the blank takes 10.28 c.c. and the sample under estimation 4.48 c.c. of 0.009207 N sodium thiosulphate, then the equivalent for N/100 thiosulphate is $(10.28 - 4.48) \times 0.9207$ c.c.: if the solution had been 0.01055 N the equivalent would be $(10.28 - 4.48) \times 1.055$ c.c. This equivalent volume when plotted against the milligrams of dextrose taken gives a graph that is not quite a straight line. From this graph a table has been prepared giving milligrams of dextrose for every variation of 0.01 c.c. N/100 sodium thiosulphate. Table V gives the actual values as determined.

The vertical column on the left gives the whole number of c.c. of N/100 thiosulphate solution and the top horizontal line the tenths of a c.c., from which can be read off the mg. of dextrose corresponding to any volume. Under the difference column is found the figures to be added corresponding to the hundredths of a c.c. Thus, 5.40 c.c. gives 1.884 mg., 5.41 c.c. gives 1.888 mg., and 5.48 c.c. gives 1.913 mg.

It is preferable to keep the amount of dextrose under estimation between the limits of about 1 and 3 mg., since below 1 mg. a small error in the titration will cause a correspondingly big error in the result. Thus, an error in titrating of 0.05 c.c. causes an error of 0.018 mg. in the result, and if only 0.5 mg. were present this represents an error of 3.6 per cent., whereas if 2.5 mg. were present the error is only 0.7 per cent.: in practice it was found difficult to obtain consistent results when the quantity exceeded 3 mg.

(d) *Sucrose.* As many of the samples of sugars under determination in this laboratory will contain sucrose, it was thought advisable to check the method against invert sugar, obtained from pure sucrose. Table VII shows the results.

TABLE VII.

Mg. Invert Sugar.	0.525	0.828	1.380	1.575	2.100	2.625	3.150
c.c. 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$	1.47	2.36	3.96	4.56	6.05	7.55	9.08

A graph plotted from these figures does not coincide exactly with that for dextrose; between 0 and 1 mg. of sugar the table for dextrose would give results low by 2 to 3 per cent.; between 1.5 and 2 mg. both graphs practically coincide; between 2 and 3 mg. the dextrose table gives results increasing from 0.5 to 2.5 per cent. too high.

For most of the work on plant tissues it is usually convenient to calculate as dextrose, since the variation for invert sugar from this is less than

the usual errors of sampling, &c. However, if more exact results are required, a table can be constructed from the results given in Table VII.

It was ascertained that sucrose itself exerted no action on the ferri-cyanide solution.

Effect of starch.

Since starch will nearly always be present in the plant tissues it was necessary to ascertain the effect of this on the iodine titration. It was found that the end point was more difficult to gauge exactly when starch was present, but that with practice correct results could be obtained: the end of the titration must be carried out slowly drop by drop, with agitation between each addition.

In conclusion it may be said that the method described is simpler and less tedious than those based on Fehling's solution, and after a little practice can be made to give consistently good results.

SUMMARY.

A rapid method for determining total carbon in dried plant tissues is described. The method consists in heating the material in a stream of oxygen in a specially designed small combustion tube and collecting the carbon dioxide formed, together with the oxygen, in a special design of Haldane type gas burette. The carbon dioxide is determined by the reduction in volume after absorption of the gas with potash.

Great saving of time is obtained by the introduction of the material into the already heated combustion tube; this allows a complete determination, including weighing, to be made in from 12 to 15 minutes. As soon as one determination is completed another can be begun at once without any cooling or heating of the tube being necessary. The actual combustion occupies 5 minutes.

The apparatus may be used to determine carbon in any type of material, but where volatile substances are used the combustion tube must be allowed to cool first.

Carbon can be determined in 0.01 grm. of substance to the nearest 0.005 mg.

By introducing an absorption tube between the combustion tube and the gas burette, and increasing the time of combustion to about 10 minutes, hydrogen also can be determined.

A method for the determination of reducing sugars is also described, consisting of a modification of the micro-method of Hagedorn and Jensen. Based on the fact that such sugars reduce alkaline potassium ferricyanide to ferrocyanide, the amounts of the various solutions have been varied from the original method so as to allow of larger amounts of sugar being

estimated, and it has been standardized against pure dextrose, a table being given for reading off directly the amount of sugar corresponding to the volume of thiosulphate used in the titration.

Invert sugar, from pure sucrose, was found to differ very slightly in reducing power from dextrose.

Sucrose, as such, has no reducing effect on the solutions. Starch renders the end point more difficult of exact determination, but some practice with the method overcomes this difficulty.

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On the Effect of Previous Treatment with Salt Solutions on the Subsequent Outward Diffusion of Electrolytes from Plant Tissue.

BY

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With three Figures in the Text.

WHILE carrying out experiments on the absorption of salts from dilute solutions by discs of carrot tissue, it was observed that in the case of tissue immersed in dilute (0.0002 M) calcium chloride the electrical conductivity of the circumambient solution was less after twenty-four hours than in controls where conductivity water was substituted for the salt solution. This suggested that calcium chloride was in some manner preventing or retarding the escape of electrolytes from the tissue. Experiments were therefore undertaken to test the effect of previous treatment with various salts on the subsequent outward diffusion of electrolytes into conductivity water.

METHOD.

The tissues used in this work were carrot root, beetroot, and potato tuber. The method of preparing the tissue was essentially the same as that used by Jørgensen and Stiles (7) in their researches. Cylinders of tissue 2.0 cm. in diameter were cut out with a cork-borer and sectioned into discs 1 mm. in thickness by means of a hand microtome. Immediately on cutting the discs were transferred to conductivity water.

All the discs for any one experiment were cut at the same time, about 360 being required for each experiment.

In order to remove the electrolytes liberated from the cut and injured cells, the discs were washed in repeated changes of conductivity water over a period of about twenty-four hours. At the end of this time the discs were divided into four different lots of about 90 each. For further treatment four solutions were used, namely:—

- (1) 0.04 M lanthanum chloride.
- (2) 0.04 M calcium chloride.
- (3) 0.04 M potassium chloride.
- (4) Conductivity water.

A single lot of about 90 tissue discs was then placed in 200 c.c. of each of these solutions, the tissue remaining there for several hours. After treatment in these solutions each lot of discs was washed in repeated changes of conductivity water.

After washing the discs were transferred to bottles each containing 60 c.c. of conductivity water. In making the transfer the discs were handled by perfectly clean hands which had been washed finally in conductivity water. Before being placed in the bottles, the discs were shaken free, as far as possible, from adhering water. Each bottle finally received 25 discs. From each lot of about 90 tissue discs three sets of 25 each were taken, so that the effect of each treatment on subsequent exosmosis was tested in triplicate. In each experiment 12 bottles were used altogether. Each bottle was furnished with a tightly fitting rubber bung.

The stoppered bottles containing the tissue were then placed on a gentle shaker operating in a constant temperature water bath kept at $20^{\circ}\text{C} \pm 1^{\circ}$.

At intervals 20 c.c. of water were temporarily removed from each bottle with a pipette and the electrical conductivity determined, the water being returned to its bottle after the determination.

Increase in conductivity may be taken as giving a rough measure of the outward diffusion of electrolytes. This method has been used by a number of workers, notably True and Bartlett (8), Stiles and Jørgensen (6), S. C. Brooks (2), and Blackman and Paine (1), to follow the exosmosis of electrolytes from plant tissue.

In making the determinations of electrical conductivity the resistance was measured with a Kohlrausch apparatus. Platinum-grey electrodes (9) were used instead of platinum-black electrodes, since in this way the adsorption effects which, when using platinum-black interfere with exact determinations in dilute solutions, are avoided.

In each experiment all the determinations of electrical conductivity were made at exactly the same temperature (21°C).

The same pair of electrodes consisting of two platinum discs 2 cm. in diameter placed 0.6 cm. apart were used throughout. With these electrodes the conductivity water used gave a slight conductivity of 2.1 in the arbitrary units employed.

RESULTS.

(1) *Carrot root tissue.*

Discs of tissue were immersed in the four test solutions for three hours and were subsequently washed in numerous changes of conductivity water for two hours before starting the exosmosis observations. The results are given in Table I.

TABLE I.

Diffusion of Electrolytes from Carrot Tissue into Conductivity Water following Treatment with Various Solutions.

Tissue previously treated with :	Experiment No.	Conductivity, in arbitrary units, after :		
		2.83 hours.	19.5 hours.	27.5 hours.
0.04 M LaCl ₃	1	9.2	26.8	36.1
	2	9.3	24.9	32.9
	3	8.3	24.9	34.6
	Mean	8.9	25.5	34.5
0.04 M CaCl ₂	4	8.9	21.0	23.7
	5	9.5	22.0	25.6
	6	9.1	23.4	28.1
	Mean	9.1	22.1	25.8
0.04 M KCl	7	33.4	79.0	84.7
	8	33.3	73.4	76.5
	9	35.2	78.0	84.1
	Mean	33.9	76.8	81.7
Conductivity water	10	15.4	41.1	43.1
	11	14.4	38.2	44.5
	12	13.5	36.0	41.5
	Mean	14.4	38.4	43.0

The mean values are plotted in Fig. 1.

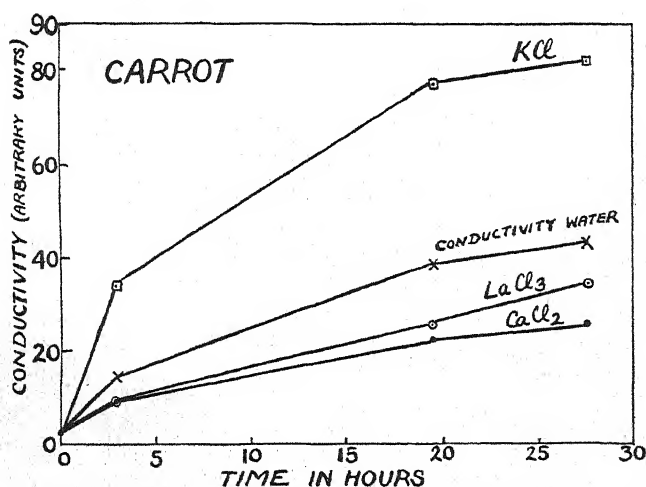


FIG. 1. Effect of previous treatment with lanthanum chloride, calcium chloride, potassium chloride, and conductivity water on the subsequent exosmosis of cell electrolytes from carrot root tissue into conductivity water. Each point represents the mean of three determinations.

(2) *Beetroot tissue.*

Discs were immersed in the test solutions for 110 minutes and subsequently washed in many changes of conductivity water over a period of 20 minutes before starting the experiment. The results are given in Table II.

TABLE II.

Diffusion of Electrolytes from Beetroot Tissue into Conductivity Water following Treatment with Various Solutions.

Tissue previously treated with :	Experiment No.	Conductivity, in arbitrary units, after :	
		3.25 hours.	7.75 hours.
0.04 M LaCl	1	17.3	31.9
	2	16.9	29.6
	3	15.2	26.8
	Mean	16.4	29.4
0.04 M CaCl	4	14.3	21.2
	5	14.9	20.1
	6	13.7	20.1
	Mean	14.3	20.4
0.04 M KCl	7	32.3	46.0
	8	33.6	48.9
	9	28.8	41.5
	Mean	31.5	45.4
Conductivity water	10	19.8	30.6
	11	21.0	31.6
	12	23.2	36.5
	Mean	21.3	32.9

The mean values are plotted in Fig. 2.

(3) *Potato tuber tissue.*

Discs were immersed in the test solutions for 90 minutes, then washed in many changes of conductivity water for a period of 30 minutes before starting the exosmosis experiment. The results are given in Table III.

Using the formulae given by Fisher (3, p. 107) for the comparison of two means, the average values for conductivity following treatment with salts was compared with the corresponding means of the values obtained following treatment with water. With one exception, the difference is in each case significant if, as is usual, $P = 0.05$ is regarded as the limit of significance. The one exception is to be found in Table II, where 7.75 hours after the commencement of the experiment the difference between the means for exosmosis from tissue previously treated with lanthanum chloride and tissue previously treated with water is not significant.

DISCUSSION.

In all the three tissues examined, previous treatment with potassium chloride solution produced subsequent increased exosmosis when the tissue was transferred to pure water. This increase, as compared with tissue

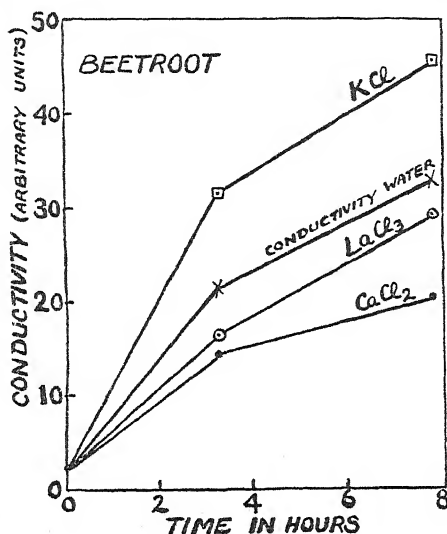


FIG. 2. Effect of previous treatment with lanthanum chloride, calcium chloride, potassium chloride, and conductivity water on the subsequent exosmosis of cell electrolytes from beetroot root tissue into conductivity water. Each point represents the mean of three determinations.

treated with conductivity water, may have resulted from the increase of total electrolytes in the tissue during the period of immersion in the salt solution. That this, at any rate in the case of potato, is not the real cause of the increased outward diffusion produced by treatment with potassium chloride, is indicated by the work of Stiles and Jørgensen (7) who showed clearly for this tissue that, in a dilute solution of potassium chloride, there is increased exosmosis as compared with that taking place into pure water.

On the other hand, in all the three tissues, treatment with calcium chloride solution produced a very definite decrease in the subsequent rate of exosmosis of electrolytes as compared with tissue treated with water alone. The same is generally true for treatment with lanthanum chloride. It would seem, therefore, that both calcium chloride and lanthanum chloride have the effect of reducing the permeability of the plasma membrane to electrolytes within the cell.

The present writer, at the time of making these experiments, was unaware of somewhat similar experiments carried out by Brooks (2) on the outward diffusion of electrolytes from the tissue of the scape of *Taraxacum officinale* into pure water, following treatment with sodium

chloride, calcium chloride, cerium chloride, and water. Brooks obtained results agreeing very closely with those reported above, namely an increase in exosmosis induced by potassium chloride and a decrease in exosmosis induced by calcium chloride and cerium chloride as compared with exosmosis from tissue treated with distilled water.

TABLE III.

Diffusion of Electrolytes from Potato Tuber Tissue into Conductivity Water following Treatment with Various Solutions.

Tissue previously treated with :	Experiment No.	Conductivity, in arbitrary units, after :		
		2.5 hours.	5.5 hours.	8.5 hours.
0.04 M LaCl	1	27.0	57.7	85.1
	2	23.1	48.1	73.1
	3	23.0	50.0	76.3
	Mean	24.3	51.9	78.1
0.04 M CaCl	4	37.9	59.2	76.3
	5	36.6	59.7	74.2
	6	36.3	58.4	73.9
	Mean	36.9	59.1	74.8
0.04 M KCl	7	67.6	114.5	143.3
	8	67.3	111.8	138.6
	9	72.8	118.3	148.1
	Mean	69.2	114.8	143.3
Conductivity water	10	45.9	77.3	90.8
	11	47.8	73.9	88.3
	12	52.2	84.1	101.2
	Mean	48.6	78.4	93.4

The mean values are plotted in Fig. 3.

The results, both of Brooks and of the writer, seem to point to certain chlorides (calcium chloride, lanthanum chloride, and cerium chloride) as having an opposite effect on the plasma membrane to that produced by other chlorides (potassium chloride and sodium chloride). In all probability the active agent is the cation. The chlorides of the bi- and trivalent cations used produce a decrease in the permeability of the plasma membrane for cell electrolytes, while the chlorides of the monovalent cations produce an increase in the permeability.

Brooks notes that the decrease produced by cerium chloride in the subsequent outward diffusion of electrolytes from *Taraxacum* tissue is only temporary and there is some indication that this is also true in the case of lanthanum chloride acting on the storage tissues examined by the writer.

Osterhout (5) has shown that lanthanum chloride, calcium chloride, and manganese chloride have the immediate effect of increasing the electrical resistance of living *Laminaria* tissue, while potassium chloride and sodium chloride produce an immediate decrease. If we are justified

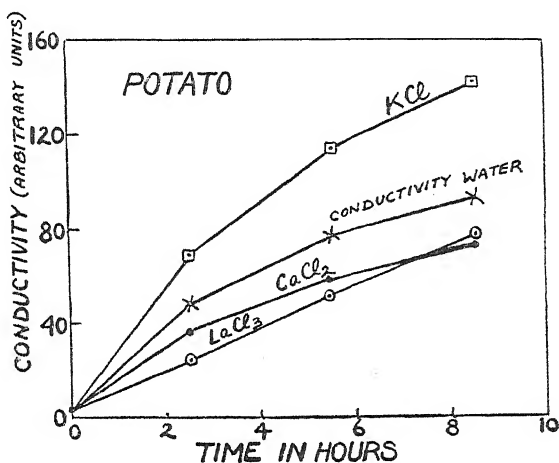


FIG. 3. Effect of previous treatment with lanthanum chloride, calcium chloride, potassium chloride, and conductivity water on the subsequent exosmosis of cell electrolytes from potato tuber tissue into conductivity water. Each point represents the mean of three determinations.

in interpreting electrical conductivity of the tissue as a measure of the permeability of the protoplasm to electrolytes, then the results of Osterhout fit in very well with the data reported in this paper.

That calcium ions produce an effect on the state of the protoplasm opposite to that produced by potassium and sodium ions is also clearly indicated by recent work on the viscosity of protoplasm. Heilbrunn (4), using the centrifuge method with *Arbacia* eggs and with the protozoan, *Stentor*, finds that the calcium ion tends to decrease and the sodium and potassium ions to increase the viscosity of protoplasm.

SUMMARY.

Outward diffusion of electrolytes from storage tissue, as measured by increase in the electrical conductivity of the circumambient solution, is markedly influenced by previous treatment of the tissue with various salts. As compared with tissue treated with pure water, calcium and lanthanum chloride reduce while potassium chloride increases the outward diffusion of electrolytes.

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Suction-Pressure Gradients and the Measurement of Suction Pressure.

BY

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THE earlier workers who attempted an analysis of water movement in plant tissues considered that the passage of water from cell to cell was determined by differences in the osmotic pressure of the cell contents. Ursprung and Blum (13), however, showed that the direction of the gradient of osmotic values was often inconsistent with this view, and pointed out that such movement must be controlled by the gradient of *suction pressure*, which is the difference between the osmotic pressure of the contents and the opposing pressure exerted by the more or less extensible wall.

This conception was a great advance upon previous views, and has deservedly passed unchallenged. In consonance with the importance of the subject Ursprung and Blum (4-18) have put forward of recent years a large body of data relating to suction pressure in the cells of different plants. In general these data may be said to have met with general acceptance, though it has been pointed out by Beck (1) and Walter (20) that they raise certain difficulties.

The data, perhaps, of most physiological interest are those of the suction-pressure gradients which should control the movement of water in tissues, as, for example, the passage of water across the cortex of the absorbing root, or the movement of water in the tissues of the transpiring leaf.

It has apparently been overlooked that the gradients of suction pressure which are assumed, no doubt rightly, to be the causal factor in such water movements, are *dynamic gradients* (*flux gradients*). If, for example, as in one of Ursprung and Blum's observations (18), there is a difference of 2.3 atmospheres between the piliferous layer of the root of *Vicia faba* and the sixth cortical cell, then that is the gradient of pressure required to bring about the flow of water through the cortex at the particular rate at which such flow is proceeding. No doubt if the flow is a steady one there would be a steady gradient of suction pressure across these cells, and the cells would be in a state of equilibrium, but it would

be a *dynamic equilibrium*, not a *static one*. It is obvious that anything which alters the rate of flow of water through these cells will alter this dynamic equilibrium. If the rate of flow is increased as by a bigger 'pull' from the transpiring leaves, there must be a new dynamic equilibrium with a higher gradient of suction pressure. If transpiration lessens there will, presumably, be a lower rate of movement across the cortex of the root, and so a new equilibrium will be set up with a smaller gradient of suction pressure. If the flow of water through the tissue is stopped—as by cutting off both supply and withdrawal—there will *no longer be a dynamic equilibrium*, and the tissues will proceed to pass into a condition of *static equilibrium*, i.e. the cells will begin immediately to gain or lose water from one another until they all have the same suction pressure.

Now in the process of measurement of the suction pressure of the cells of a tissue, a portion of the tissue is placed in paraffin and then sections are cut and mounted in paraffin and transferred to an osmotic solution so that the suction pressure may be determined. The removal of a portion of a plant organ such as a root or a leaf and its submersion in paraffin oil will immediately reduce to zero both the supply of water to, and the loss of water from, the tissues. *The dynamic relationships of the cells on which the suction-pressure gradient depends will be completely upset directly the tissue is removed from the plant, and as in paraffin there is neither gain nor loss of water the cells will immediately begin to pass into a static equilibrium, and they should in a short time all reach the same suction pressure.*

It is evident that it is impossible to measure *normal* suction-pressure gradients by the method of Ursprung and Blum, in which the tissues are removed from the plant, and the cells, in which the gradient is to be determined, are left in contact with one another for a considerable period.

It is somewhat remarkable that this fundamental difficulty in the application of the Ursprung and Blum method to the determination of suction-pressure gradients does not seem to have been pointed out before. These gradients are dynamic gradients, and they must alter profoundly and rapidly when the dynamic water relationships of the cells concerned are affected. Any suction pressures measured by the method in question can have little relationship to the suction-pressure gradients existing in normal tissues attached to the plant.

Beck(1) pointed out one of the anomalous results obtained by Ursprung and Blum, namely, that in the leaf of *Hedera Helix* the upper and lower epidermis cells have a *lower* suction pressure than that of the neighbouring mesophyll cells from which presumably they obtain water, i.e. the gradient is in a *reverse* direction to that expected. With reference to this anomaly Ursprung(6) states: 'The exceptional position which the epidermal tissues occupy in the series can be understood only if it be assumed that these cells obtain their supply of water laterally from the principal veins, and function

as reservoirs of water.' This assumption can hardly be accepted. Unless the lower wall of the epidermal cell is impermeable to water such a gradient could not continue to exist for any considerable period in a cut-off leaf. As shown later in this paper, such an anomalous result is an almost inevitable consequence of the method of experimentation.

The simple physical considerations already outlined indicate that excised tissues in paraffin should sooner or later show the same suction pressure in all the cells. In the Ursprung and Blum method a period of twenty to thirty minutes is allowed for the cells to attain equilibrium with the sugar solution employed to determine the osmotic pressure which will just balance the suction pressure. If, however, such a period is sufficient to allow cells to attain equilibrium with an external solution, it should at least be sufficient to modify profoundly the normal gradient, even if it does not bring about its abolition.

As Ursprung and Blum in numerous cases record marked and persistent differences in suction pressure between the cells of excised tissues in paraffin, a study of the method employed in obtaining such data was undertaken.¹ It may be stated at once that *a flaw hitherto overlooked has been discovered in the method.*

The flaw in question invalidates every measurement of suction pressure made on sections by the method first put forward in 1916, and modified in minor, though technically important, details in subsequent papers. It shows that the results obtained *do not give a measure even of the suction pressure of the cells at the time the sections are cut from the piece of tissue immersed in paraffin.* The inadequacy of the method—apart from the difference produced by change of suction pressure with time in an excised tissue—is due to the effect of the exuding sap of the cells laid open by sectioning on the *intact* cells used for measurement. Ursprung and Blum are careful to state that they worked with intact cells only; it would, of course, be impossible to measure the suction pressure of a ruptured cell. They emphasize the necessity of obtaining *thin* sections which will give a clear view of the cells; in the most satisfactory sections the proportion of intact cells is therefore smallest. *The assumption that an intact cell retains its suction pressure unaltered when the surrounding cells are laid open cannot be accepted.* One may consider the hypothetical case of two neighbouring palisade cells with the same suction pressure. Assume that each has a similar wall pressure, then their suction pressure will be smaller than the osmotic pressure of their contents by the amount of the wall pressure. If one of the cells is ruptured the released contents will be capable of exerting their full osmotic pressure. This osmotic pressure will be greater than the suction pressure of the intact cell, and the contents coming in contact with

¹ Oppenheimer (8) points out many possible sources of error in the Ursprung and Blum technique, but he considers that with practice these can be reduced to a minimum.

the latter (as they normally would in the application of the method) will draw water from the intact cell and *its suction pressure will be raised*.¹

The greater the number of ruptured cells of the same osmotic pressure contributing to the quantity of sap in contact with the intact cell, the higher will its suction pressure be raised above the normal value, and the nearer it will approach the full value of the osmotic pressure of its contents.

Experimental Demonstration of the Wound-Effect in the Determination of Suction Pressure by the Method of Sections.

Elimination of the wound factor.

It is possible to obtain the cells of certain leaves in a state suitable for fairly easy determination of the suction pressure *without sectioning the tissues*, and so without submitting intact cells to the action of the osmotic fluids from ruptured cells.

The lower epidermis of the leaves of certain dicotyledons can be stripped off, following a small incision with a sharp scalpel; attached to the epidermis is a smaller or larger number of mesophyll cells, which in many cases appear perfectly uninjured. The epidermis of many monocotyledonous leaves can be obtained in the same way with one or two layers of mesophyll cells attached. The veins can also be torn from some monocotyledonous leaves in such a manner that a number of mesophyll cells separate with them.

The suction pressure of mesophyll cells obtained in this way has been compared with the suction pressure of similar cells determined by the use of sections according to the method usually employed.

Crocus leaf. The lateral vein of a crocus leaf was rapidly stripped away and mounted in paraffin. The strip was divided lengthwise into three or four pieces, and the suction pressure of the mesophyll cells in each preparation estimated in the usual way by determining the change in area of the cells in sugar solutions.

At the same time as the strip was taken a part of the same leaf on the further side of the main vein, at the same level on the leaf, was removed, placed in paraffin, and sectioned. The suction pressure of the mesophyll cells of the section was again estimated.²

¹ In the simple case of two cells the suction pressure of the intact cell, after equilibrium is reached, should give a value higher than the normal pressure by a quantity equal to half the wall pressure ($1/2 W$), if, in Ursprung's terminology, $S_z = S_i - W$ (suction pressure of the cell = suction pressure (i. e. osmotic pressure) of the cell contents less wall pressure). On the other hand, the osmotic pressure of the medium surrounding the intact cell will be reduced by $1/2 W$.

² When attempting to determine suction pressure it was found impossible to work satisfactorily in a laboratory in which the air was contaminated with coal gas. In the presence of this gas death of the cells occurred very rapidly. This may perhaps explain the negative results obtained by some workers when investigating suction pressure.

TABLE I.

Comparison of Values obtained by Ursprung and Blum's Method and by the Improved Method of Measuring Suction Pressure.

Mesophyll of Crocus.

	Sugar conc.	Cell type.	Area.		% Change.
			Paraffin.	Sugar sol.	
LEAF 1.					
<i>Improved Method:</i>					
Strip A	0.23 M	Middle mesophyll	90	95	+6
			88	92	+5
			99	105	+6
			87	93	+7
			100	107	+7
			175	185	+6
Strip B	0.25 M	1st palisade	88	81	-8
			142	124	-12
			128	120	-6
			120	111	-8
			119	110	-7
			98	90	-8
			153	139	-9
			142	131	-8
		2nd palisade	138	132	-4
			94	89	-5
			109	102	-6
			92	88	-4
			98	89	-9
			84	77	-8
			100	95	-5
<i>Old Method:</i>					
Section A	0.35 M	1st palisade	81	88	+8
			103	109	+6
			115	124	+7
		2nd palisade	68	71	+4
			82	84	+2
			87	89	+2
Section B	0.36 M	1st palisade	135	140	+3
			137	139	+1
			125	127	+1
		2nd palisade	87	89	+2
			91	94	+3
			76	80	+5
			135	141	+4
		Middle mesophyll	107	107	0
		Section C	0.37 M	1st palisade	85
99	96				-3
94	87				-7
84	82				-2
2nd palisade	122			113	-7
	111			106	-4
	82			77	-6
	113			106	-6
Middle mesophyll	115			113	-1

TABLE I (continued).

LEAF 4.					
	Sugar conc.	Cell type.	Area		% Change.
			Paraffin.	Sugar sol.	
<i>Improved Method:</i>					
Strip A	0.23 M	1st palisade	111	114	+3
			99	102	+3
			105	107	+2
			100	108	+8
		2nd palisade	68	73	+7
			81	84	+3
Strip B	0.25 M	1st palisade	103	98	-5
			129	118	-9
			138	130	-6
Section A	0.35 M	1st palisade	93	97	+4
			88	96	+9
			68	70	+3
		2nd palisade	48	52	+8
			77	84	+9
			63	66	+5
			77	81	+5
		Regular mesophyll	126	133	+10
			52	58	+11
			<i>Old Method:</i>		
Section B	0.36 M	Mesophyll cells at edge of leaf	60	62	+3
			66	68	+3
			52	56	+8
			36	38	+6
			70	76	+8
			61	65	+6
			46	52	+13

In all, seven leaves were investigated by the two methods. The results for leaves 1 and 4 are given in full in Table I, while the results as a whole are summarized in Table II. The areas are given in arbitrary units.

Table II shows that in every case the method of Ursprung and Blum gives far higher values than are given by the improved method. The results indicate clearly the effect of ruptured cells on intact ones and demonstrate the inaccuracy of the section method.

Ursprung and Blum (12), Ursprung and Hayoz (19), and Hayoz (2), in their determination of the changes of suction pressure and its distribution in leaves separated from the plant and allowed to wither, encountered the rise in suction pressure which wounding entails. Each measurement necessitated a new cutting of the leaf, and this induced an increased suction pressure in the 20-30 palisade cells adjoining the wound. Therefore they took each series of sections some distance from the surface exposed by the previous wound. They say, (12) 'Da jede Messung eine Verletzung des Blattes erfordert, war zuerst der Einfluss einer solchen Verletzung auf die Saugkraft

festzustellen. Eine grössere Versuchsreihe . . . mit Epidermiszellen und Palisaden . . . ergab, dass vergleichbare Palisaden (jeweils die 20. Palisade von demselben Nerv entfernt) in nächster Nähe der Wunde ihre Saugkraft erhöhen, dass aber von der 30. oder 40. Palisade (von der Wunde gerechnet) an, d. h. ca. 1/2 mm. der Wunde entfernt, die Saugkraft wieder normal war.'

TABLE II.

Summary of Values of the Suction Pressures of the Mesophyll Cells of Crocus obtained by the Original and the Improved Methods.

Leaf No.	Suction Pressure. (Atmospheres).	
	Method of Ursprung and Blum.	Improved method.
I.	> 9.6	> 6.1
	> 9.9	—
	< 10.2	< 6.7
II.	> 9.6	> 6.1
	< 10.2	< 6.7
III.	> 9.6	> 6.1
	> 10.2,	—
	—	< 6.7
IV.	> 9.6	> 6.1
	> 9.9	—
	—	< 6.7
V.	< 9.6	> 6.1
	—	< 6.7
VI.	< 9.6	< 6.1
	—	> 6.7
VII.	< 9.6	< 6.1
	—	> 6.7

They allow that wounding has an effect on suction pressure measurements, but do not realize the significance of the observation in the interpretation of the mass of data they have obtained. As in the work on the withering leaf numerous sections had to be cut so as to obtain the material for a series of tests the number of wounded cells must in the aggregate have been large, and this would result in a relatively large volume of released sap which would markedly increase the suction pressure of those cells in the unsectioned part of the leaf with which it came in contact. The authors admit that the effect reached to a depth of 30 to 40 palisade cells from the cut surface.

Nature of the Apparent Suction-Pressure Gradients.

(1) *The reversed gradient between epidermis and palisade.*

The osmotic concentration of the released sap which acts on the intact cells will depend on the type of cell which is ruptured, and this again will

depend on the direction of the section. On this basis the *reverse gradient* observed in Ivy leaf by Ursprung and Blum (i.e. the low suction pressure of the epidermal cells and the high suction pressure of the adjacent mesophyll cells) can be explained. For a study of the epidermal cells a surface section will be cut providing only a *small* quantity of sap from the injured palisade cells to raise the suction pressure of the epidermal cells. For a study of the palisade cells a transverse section will be cut which will be likely to release a *large* amount of sap and so raise the suction pressure of the palisade cells considerably.

(2) *The Ursprung and Blum gradient in the mesophyll.*

Ursprung and Blum's apparent gradients in suction pressure of the palisade cells *extended from a main vein for some 140 cells*, although it is generally considered that the ultimate water source is the vein *ending*, and that no cell is more than a few cells distant from such a vein tip. The liberated sap to which cells in transverse sections are subjected will vary in concentration according to the relative amounts of sap contributed from cut epidermal and cut palisade cells. The sap of the palisade cells has a higher osmotic concentration than that of the epidermal cells, so the sap of the cut palisade cells will be diluted, and its effect lessened, to some greater or less degree, by the epidermal cell contents.¹ The different proportions of these two saps in different sections must lead to anomalous results. The lower suction pressure observed by Ursprung and Blum in cells from sections including vein tissue was probably produced by the diluting action on the contents of the ruptured cells of the water contained in the veins. Also water would be withdrawn from the tracheids when the tension in the water columns was destroyed by cutting off the leaf.

The Equalization of Suction Pressures in the Detached Leaf.

The maintenance of gradients of suction pressure in leaves for the length of time necessary for their demonstration by the method of Ursprung and Blum has been shown on general grounds to be hardly possible. Experimental work has confirmed these doubts by failure to observe such gradients.

The most striking gradients were described by the previous workers (6) in palisade cells of the leaf at increasing distances from main veins. A study of the leaf of *Iris* was therefore undertaken by the modified method

¹ In a transverse section the released contents of cut epidermal cell contents tend to *lower* the measured suction pressure of the palisade cells, but in a hypothetical case in which the suction pressures of epidermal and palisade cells are previously in equilibrium and the epidermal cells alone suffer mechanical injury the suction pressure of the palisade cells would be *increased*, since they must now attain equilibrium with the osmotic concentration of the epidermal cell contents, a value greater than their suction pressure value by the amount of the wall pressure in the uninjured epidermal cells.

in which *stripped* portions of the leaf, instead of *sections*, are employed. In this leaf the epidermis can easily be peeled off. It retains one row of mesophyll cells, excepting along the course of the veins. The approximate suction pressure of these mesophyll cells was determined, as shown in Table III.

TABLE III.

Iris Leaf. Suction Pressure of the Mesophyll Cells in Surface Strips.

Strip.	Sugar conc.	Area.		% Change.
		Paraffin.	Sugar.	
1.	0.35 M	113	104	-7.8
		98	90	-8.1
		157	145	-7.6
		126	122	-3.1
		117	108	-7.6
		121	113	-6.6
2.	0.33 M	137	131	-4.3
		117	114	-2.5
		137	124	-9.4
		173	169	-2.3
		191	179	-6.2
3.	0.31 M	244	246	+0.8
		167	170	+1.8
		130	130	—
		198	198	—
		175	175	—
4.	0.29 M	117	122	+4.1
		116	123	+7.0
		174	181	+4.0
		109	113	+3.6

The data given in Table III show that the suction pressure of the mesophyll cells attached to the epidermal strips lies between 0.33 M and 0.31 M cane sugar.

In order to test for the occurrence of gradients of suction pressure in these mesophyll cells the behaviour of a series of 41 cells lying between the courses of two veins was studied by observing their change in area when placed in 0.33 M cane sugar. A marked gradient of suction pressure should show itself by a marked and graduated difference in the percentage change of area of cells occupying different positions in the series. The results are given in Table IV.

It is clear from the results of Table IV that these preparations supply no evidence for the existence of gradients of suction pressure in the mesophyll cells lying between two veins. *The reaction of the cells is practically the same throughout the series.*

Since negative results were obtained with the leaf of *Iris*, similar observations were undertaken, using the leaf of *Saxifraga umbrosa*. Mesophyll

cells attached to peeled strips of the lower epidermis were employed, and the cells lying between the courses of two veins were studied as shown in Table V.

TABLE IV.

Iris Leaf. Reaction of Mesophyll Cells (1-41) Stretching from One Vein to Another in 0.33 M Sugar Solution.

Cell No.	Area		% Change.	Cell No.	Area.		% Change.
	Paraffin.	Sugar.			Paraffin.	Sugar.	
1.	143	138	+3.4	21.	134	128	+4.4
2.	152	148	+2.6	22.	196	185	+5.6
3.	206	197	+4.3	23.	134	130	+2.2
4.	174	165	+5.1	24.	151	143	+5.3
5.	144	139	+2.7	25.	220	211	+4.0
6.	113	109	+3.5	26.	164	160	+2.4
7.	133	128	+3.7	27.	144	140	+2.7
8.	155	145	+6.3	28.	146	139	+4.7
9.	115	111	+3.4	29.	107	102	+4.6
10.	121	117	+3.3	30.	109	104	+4.6
11.	139	135	+2.8	31.	173	165	+4.5
12.	130	127	+2.3	32.	154	148	+3.2
13.	195	185	+4.9	33.	170	165	+2.9
14.	111	105	+5.4	34.	173	165	+4.5
15.	166	159	+4.2	35.	174	164	+4.5
16.	137	128	+6.5	36.	175	169	+3.4
17.	132	126	+4.5	37.	163	156	+4.3
18.	195	185	+4.9	38.	130	124	+3.0
19.	94	90	+4.2	39.	155	151	+2.5
20.	130	126	+3.0	40.	147	143	+2.6
				41.	152	146	+3.9

TABLE V.

Saxifraga umbrosa. Reaction of Mesophyll Cells Stretching from One Vein to Another.

Preparation.	Sugar conc.	Cell No.	Area		% Change.
			Paraffin.	Sugar.	
I.	0.23 M	1.	239	252	+5.9
		2.	200	219	+9.5
		3.	234	245	+4.7
		4.	198	230	+16.1
		5.	414	451	+8.9
		6.	232	237	+2.1
		7.	293	313	+6.8
		8.	293	302	+3.0
		9.	236	250	+6.3
		10.	452	482	+6.6
		11.	187	197	+5.3
		12.	215	250	+16.2
		13.	293	317	+8.1
		14.	222	234	+5.4
		15.	233	249	+6.8
		16.	98	102	+4.0
		17.	84	88	+4.7

Preparation.	Sugar conc.	Cell No.	Area		% Change.
			Paraffin.	Sugar.	
		18.	175	184	+ 5.1
		19.	180	194	+ 7.7
		20.	138	144	+ 4.3
		21.	367	394	+ 7.6
		22.	183	198	+ 8.2
		23.	90	102	+ 13.3
		24.	234	264	+ 12.3
II.	0.25 M	1.	453	477	+ 5.3
		2.	244	266	+ 9.0
		3.	309	322	+ 4.2
		4.	384	412	+ 7.3
		5.	254	274	+ 7.9
		6.	303	323	+ 6.6
		7.	330	346	+ 4.9
		8.	205	215	+ 5.0
		9.	202	220	+ 9.0
		10.	198	208	+ 5.0
		11.	163	175	+ 7.3
		12.	143	149	+ 4.2
		13.	412	420	+ 2.0
		14.	269	279	+ 3.5
		15.	264	278	+ 5.3
		16.	187	207	+ 10.7
		17.	248	277	+ 11.7
		18.	351	379	+ 7.9
		19.	356	370	+ 3.9
		20.	375	395	+ 5.3
		21.	299	308	+ 3.0
		22.	290	299	+ 3.0
		23.	222	244	+ 9.0
		24.	377	405	+ 7.4
III.	0.27 M	1.	403	363	- 10.0
		2.	546	493	- 9.5
		3.	418	344	- 17.2
		4.	490	459	- 6.3
		5.	576	537	- 6.7
		6.	476	432	- 9.2
		7.	493	422	- 14.4
		8.	557	482	- 13.4
		9.	324	308	- 4.9

In the 57 mesophyll cells of *S. umbrosa* examined there is some irregularity, about ten of the cells tabulated showing considerably greater or smaller changes than the rest, but in general the cells show a fairly uniform suction pressure. *There is no evidence at all of a gradient of pressure.*

In order to obtain further evidence of the attainment of an equilibrium of suction pressure in the cut-off leaf an investigation was made of the various cells which may be found attached to a lateral vein stripped from a *Crocus* leaf in the manner described. These were subjected to solutions of 0.22 M and 0.25 M cane sugar, which differ in osmotic pressure by only 0.8 atmosphere, with the results shown in Table VI.

TABLE VI.

Crocus Leaf.

	Sugar conc.	Cell type.	Area.		% Change.		
			Paraffin.	Sugar.			
LEAF 1.							
Strip A	0.22 M	Elongated lower mesophyll	123	126	+2		
			172	178	+3		
			145	150	+3		
			157	167	+6		
			138	146	+6		
		Cells abutting on vein	68	74	+9		
			79	83	+5		
			75	79	+5		
			77	88	+14		
			75	81	+9		
			84	90	+7		
			53	59	+11		
			46	48	+4		
			51	54	+6		
			40	44	+10		
			40	45	+12		
			63	69	+9		
			87	96	+10		
			58	65	+12		
			58	62	+7		
Strip B	0.25 M	2nd palisade	81	77	-5		
			101	97	-4		
			90	83	-8		
			84	77	-8		
			84	79	-6		
		Elongated upper mesophyll	177	164	-7		
			122	111	-8		
			102	93	-7		
			120	116	-4		
			125	120	-4		
			157	147	-6		
			103	98	-5		
			100	95	-5		
			113	100	-8		
			81	75	-7		
		Cells against small vein	45	40	-11		
			140	126	-10		
			96	88	-8		
			130	121	-7		
			92	87	-5		
		LEAF 2.					
		Strip A	0.22 M	Mesophyll cells between two veins	117	126	+7
					105	101	+4
					127	133	+5
					102	111	+9
99	111				+12		
92	99				+8		
145	155				+7		
138	147				+6		
139	156				+16		
121	135				+11		
108	117				+8		
108	117				+8		

	Sugar conc.	Cell type.	Area.		% Change.
			Paraffin.	Sugar.	
Strip B	0.25 M	Mesophyll cells between two cells	137	126	-8
			98	89	-9
			91	84	-8
LEAF 3.					
Strip A	0.22 M	Regular mesophyll	73	78	+7
			110	119	+8
			69	72	+6
			84	86	+2
			81	87	+7
			109	116	+6
Strip B	0.25 M	Regular mesophyll	90	83	-8
			122	117	-4
			95	89	-6
			47	44	-6
			97	91	-6
			67	62	-7
			76	74	-3
			92	89	-3
			58	55	-6
			104	101	-3
			25	24	-4
			33	30	-9

The results (Table VI) show that the suction pressure of all the cells included in these preparations—palisade, the regular spongy cells between palisade and vein, the spongy cells directly adjoining the vascular sheath, and those between the vein and the lower epidermis—lies well within the limits of the two osmotic concentrations, to which the different preparations were alternately subjected.

The results obtained with the mesophyll cells of the leaves of *Iris* and of *Saxifraga umbrosa*, and with various cells of the leaf of *Crocus* clearly indicate that an approximate equilibrium of suction pressure is quickly attained by the cells of a cut-off leaf. In the case of *Crocus* the time between detaching the leaf and completing the determination was in no case more than twenty minutes, and the time was not much longer in the case of the other leaves.

The results just described, and the difference between the results obtained by the section method and the improved strip method, clearly indicate that the gradient of suction pressure observed in excised tissues by previous workers is an *illusion of experimentation* produced by differences between the suction pressure of the intact cells and the osmotic concentration of the saps of the surrounding cells which have been liberated by the method of preparation. The suction pressure quickly becomes equalized in the cells of the detached leaf, as has already been shown in this paper. The osmotic concentration of the released sap depends on the

direction and thickness of the section, the number and type of the cells included in it, the proportion of injured to uninjured cells, and the suction pressure of all these cells.

SUMMARY.

It is pointed out that the suction-pressure gradients assumed to be responsible for the movement of water through tissues are *dynamic gradients (flux gradients)*. Accordingly, if the flow of water is stopped as in a portion of a detached leaf placed in paraffin, *the gradients will rapidly disappear and equalization of suction pressure result.*

It is shown that in determining suction pressure by the method of Ursprung and Blum, in which sections are employed, the results are completely vitiated by the increase in suction pressure of the intact cells caused by contact with the sap of the cells laid open in sectioning.

The flaw in that method can be obviated by the use of *strips* of tissue instead of *sections* of tissue.

The suction pressures obtained by use of the improved method are shown to be always much lower than the anomalous pressures obtained by the Ursprung and Blum method of sections.

Study of cells of the *excised* leaves of Crocus, Iris and *Saxifraga umbrosa* brought to light no evidence of gradients of suction pressure. All tissues showed approximately the same suction pressure as was to be expected on general grounds. Such an equilibrium of suction pressure appears to be attained within a period of twenty minutes after the removal of the leaf from the plant.

In view of the published data, which appear to demonstrate the persistence—on a *a priori* considerations almost inexplicable—of suction-pressure gradients and suction-pressure differences in tissues of cut-off organs immersed in paraffin, Professor V. H. Blackman suggested to me the need for a study of the methods employed in such measurements. It is a pleasure to express my grateful thanks for his very kind help in the course of this investigation.

Postscript. Since the above was written a comment by H. H. Dixon 'The Transpiration Stream', (London, 1924) has been noticed. He states (p. 43) with reference to measurements of suction pressure by the Ursprung and Blum method: 'It is also difficult to see how it is that when the excisions are being made and the sections are being prepared, the volume of the cell does not alter. For at that moment they must be surrounded with solutions not necessarily of the concentration balancing their suction force.' The criticism is not developed further.

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An Improved Type of Moist Chamber for Studying Fungal Growth.

BY

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With two Figures in the Text.

THE study of micro-fungi largely involves the close examination of the fine structure of the conidiophore and the arrangement and mode of attachment of the conidia. These features are frequently difficult to determine by ordinary methods, particularly when the structures are very minute. Direct observation of Petri-dish cultures usually permits the use of the lower powers only, and with teased material the conidia are easily displaced. The Van Tieghem cell, or 'Hanging Drop', is useful; but it has the great disadvantage that the fungus is growing away from the cover-glass, and consequently the later stages of growth are difficult to observe under the high powers. During a preliminary survey of the moulds occurring on dairy products many fungi were isolated which proved troublesome in these respects. The following method proved very useful, and saved much time and labour.

The method.

Place a small quantity of hot medium (80-90° C.) on a sterile slide and, just before it gels, flatten by placing on the top a sterile cover-slip, using gentle pressure if necessary. The correct time for putting on the cover-slip may be judged by gently rocking the slide and noting the stage when the movement in the centre of the drop almost ceases. In about two minutes, when the medium has thoroughly set, slide the cover-glass from the disc of medium. With a sterile knife, preferably straight-edged, or better still, a safety razor blade, cut across a diameter and push half the disc to one side, leaving a space of about $\frac{1}{2}$ -1 cm. between the cut surfaces. Inoculate one of the cut surfaces and quickly cover with a sterile cover-slip. Press the cover-slip lightly on to the disc of medium until it is held by the surface moisture. Incubate in a moist atmosphere.

The method will be easily understood from Fig. 1, which shows a finished cell (*a*) in surface view, (*b*) in side view.

It will be seen that the cell is open at both ends, and consequently will tend to dry up during examination. This is of no importance for short examinations; but, should examination be carried on over a long period, it is advisable to place a piece of damp filter paper at each end of the cavity.

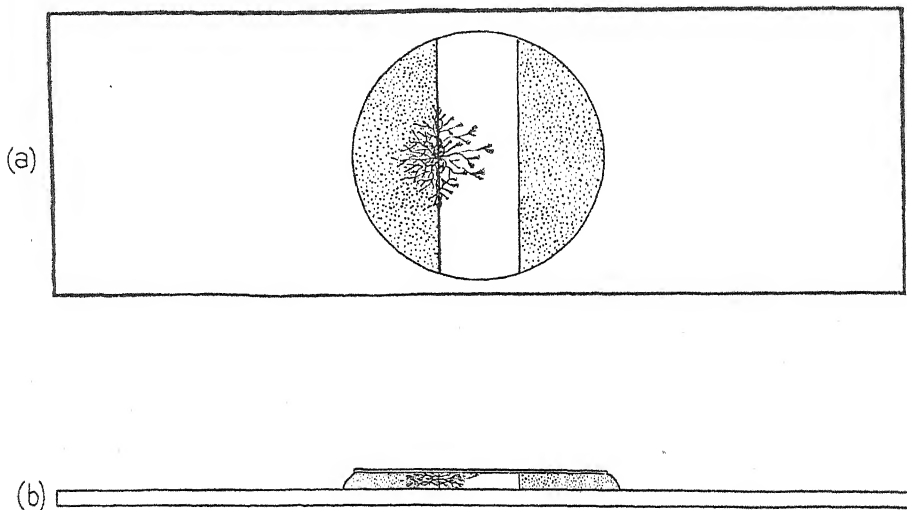


FIG. 1. Illustrating culture-cell; (a) in surface view, (b) in side view.

Gelatin media are not suitable as the cover-glass is difficult to remove and a torn disc usually results. Any agar medium may be used. A medium which gels strongly is much easier to handle, and with neutral or slightly acid media 2 per cent. agar gives good results. Very acid media may require more. A thin growth is desirable, and the agar medium most suited to the particular fungus under examination should prove most satisfactory if used as dilute as is consistent with sporulation. A 2 per cent. plain agar has been found to give good results with many organisms.

The amount of medium to be placed on the slide will naturally depend on the size of the cover-glass and the thickness required of the agar disc. About three drops gives a disc of suitable thickness with a $\frac{7}{8}$ in. cover-glass.

When the inoculum, consisting of a mass of spores, is placed on the cut edge and the cover-slip laid on, there is a tendency for some of the spores to move outwards in the film of water on the cut surface. For ordinary observation this is usually advantageous, as small clean colonies arise from the spores so displaced. When, however, a single spore culture is being set up, this liability to displacement is troublesome. The single spore should be placed a little distance behind the cut edge, in which case it does

not move. Mycelial inocula should be similarly placed in order to leave the cut edge clean.

So far as the writer's experience has gone, it is immaterial in what position the slides are placed during incubation. No trouble has been met with in the way of undesirable curvatures of the conidiophores caused by gravity or light. A point of importance is that the atmosphere of the vessel in which the slides are incubated should not be too damp, otherwise moisture tends to condense on the under surface of the cover-slip. When such condensation occurs it is necessary to wait until the excess moisture has evaporated before the slide can be examined. Furthermore, there is a tendency for hyphae to grow rapidly in this condensation moisture. When little moisture is present this surface growth is negligible, as it is possible to focus through it on to the hyphae growing freely in the cavity below.

The method possesses a great advantage in that the main direction of growth is at right angles to the line of vision, so that a lateral view of the developing structures is obtained. It is possible with the aid of the high power, or even in favourable cases with the oil immersion lens, to follow all stages from the germination of the spore to the production of a new crop of spores. The oil immersion objective may be usefully employed when a very thin cover-slip is used. It is then not only possible to examine structures closely applied to the under surface of the cover-glass, but also free conidiophores, &c., lying in the cavity below. The cover-glass is firmly attached to the agar, and the immersion oil may be safely removed without disturbing the culture. With a clear medium the submerged hyphae are visible, and any structures such as conidia, chlamydospores, &c., embedded in the medium may be studied. The habit of the colony, often an important character, particularly with species *Penicillium* and *Aspergillus*, can be examined conveniently under the lower powers. The slide, in effect, gives an undisturbed section of the colony, and the general characteristics are easily seen. Excellent photographs, showing the growth habit of several species of *Penicillium* and *Aspergillus*, have been obtained.

Fig. 2 illustrates the development of the conidiophore and spore formation of *Trichoderma lignorum* observed over a period of twelve hours. The conidia are seen to be borne singly in a drop of liquid, a group of spores being left at the tip when the drop disappears.

As a further illustration of the value of the method, a species of *Sporotrichum* may be cited. Teased material of this fungus gave no indication of the arrangement of the spores; but, when cultures were made by the above method, the actual formation of the spores was observed and the preparation was sufficiently clear for photographs of the spore head to be taken.

In general, the method has proved of great service where other methods have been unsatisfactory. Information has been acquired by direct

observation, that could either not have been obtained by ordinary methods at all or could only have been arrived at after much labour and by piecing together evidence collected from many teased-out preparations.

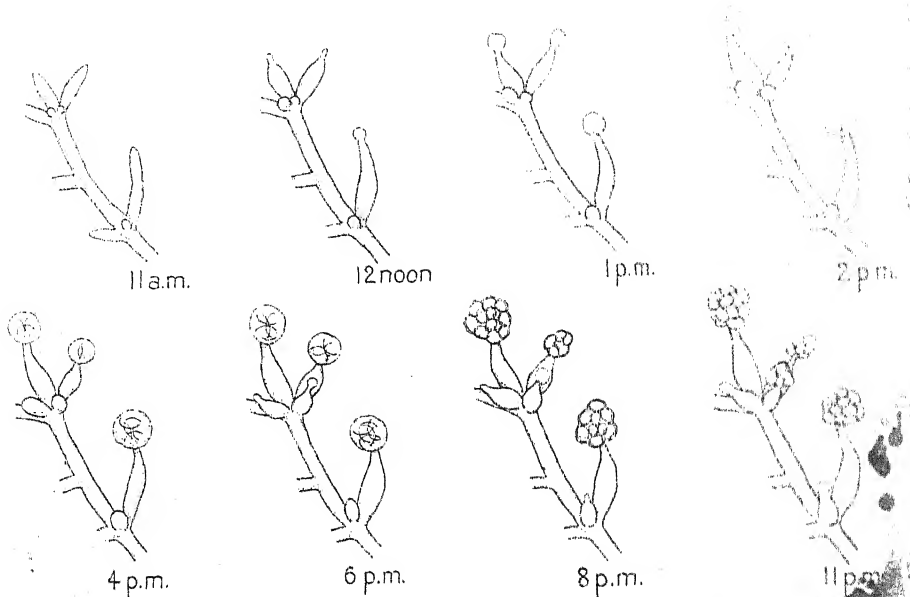


FIG. 2. Illustrating spore development in *Trichoderma lignorum* over a period of twelve hours. Camera lucida drawings. ($\times 700$ approx.)

With ordinary precautions very little trouble has been experienced with contamination from the air, although both mouldy cheese and mouldy cacao beans have been handled in the laboratory. Preparations may be kept in good condition for at least several weeks, and will serve therefore for any fungus which is not very tardy in sporulating.

That the method has wide scope is indicated by the large number of fungi which have been examined in this way by the writer. The majority of these have been isolated from butter and cheese, some from cacao beans, and a few from other sources. The list comprises species from the following genera.

Penicillium.
Aspergillus.
Gliocladium.
Paecilomyces.
Scopulariopsis.
Stysanus.
Verticillium.

Monilia.
Sporendonema.
Cladosporium.
Hormodendrum.
Alternaria.
Stemphylium.
Fusarium.

Acrostalagmus.

Cylindrocarpon.

Sporotrichum.

Trichoderma.

Botrytis.

Successful preparations have been made with these fungi, and in many cases either camera lucida drawings made or photographs taken of various stages of spore development.

In addition to its general usefulness for purposes of research, the method should be of considerable value for class demonstration. The slides are easy to prepare, and the whole process may be carried out in a few minutes.

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